CHAPTER ONE

1.0 Introduction

Iodine is a non-metallic element belonging to the halogen family in Group VIIA of the periodic table (Cooper, 2007). It is a dark purple, crystalline and lustrous solid at room temperature. It dissolves to liquid when it is heated at 113.5^0C and boils to a pinkish purple vapour at 184^0C, although it can sublime to vapour directly from the solid, depending on circumstances (Cooper, 2007).

Iodine is necessary for the synthesis of thyroid hormones; triiodothyronine (T_3) and thyroxine (T_4) (Haldiman *et al.*, 2014). The recommended dietary intake (RDI) of iodine for adults is 150µg/day. It could be obtained by consuming foods such as seaweed, milk and dairy products, iodised table salt and seafood (Küpper *et al.*, 2011).

The thyroid hormones (T_3 and T_4) are metabolic in nature. Most of the plasma pool of T_3 is derived from peripheral outer ring- or 5'-monodeiodination of T_4 (Leonard, 2005).T_3 increases almost every aspect of metabolism which includes carbohydrate utilization. It accelerates the absorption of glucose from the digestive tract, gluconeogenesis and glycogenolysis in liver cells, and glucose oxidation in liver, fat and muscle cells (Leonard, 2005).

Although the recommended intake of iodine is sufficient for the synthesis of thyroid hormones, it may be inadequate for some other functions of iodine in the body. For example, iodine is necessary for the maintenance of healthy breast tissue (Miller, 2006) because it has been linked with maintenance of the oestrogen/progesterone balance and the intake of iodine supplements have been revealed to assuage symptoms related to oestrogen dominance like fibrocystic breast disease (Ghent *et al.*, 1993). In areas where there is intake of high quantities of iodine (about 100 times more than the RDI) such as Japan, occurrence of diseases like breast cancer was the slightest when compared to other developed countries till recently and the life expectancy is the highest in

the world (Miller, 2006).

Goitrogenic foods includes foods, vegetables and grains that suppress the function of the thyroid gland, this can result to the enlargement of the thyroid gland (goitre). Examples of goitrogenic foods include; cassava and its products, peanuts, soybeans and its products and cruciferous vegetables such as broccoli, mustard, cabbage cauliflower, brussel sprouts, rutabagas kohlrabi and turnips. Soy and its products contain isoflavones which reduce thyroid hormone output by blocking the activity of the enzyme thyroid peroxidase (McCarrison, 1933) (Sharpless *et al.,*1939). Cassava tuber products contain cyanide (McPhee *et al*., 2009) which is digested to form thiocyanate. This compound can block thyroidal uptake of iodine by competing with iodine for sodium iodide symporter (NIS) (McPhee *et al*., 2009).

1.1 Statement of Research Problem

The rate of consumption of cassava (*Manihot esculenta*) and its products is on the rise especially in sub-Saharan Africa where the cultivation and consumption of cassava and cassava products have rapidly increased, tripling in the last twenty years (MediaGlobal, 2009). Almost one billion people around the world depend on cassava as a staple food especially in Africa where only maize supplies more calories (MediaGlobal, 2009). In Nigeria, it was implemented that flour for the production of bread should contain20% cassava flour. But cassava tubers have cyanide which is a powerful toxin. Most of the cyanide content of cassava can be eliminated by appropriate processing, but no matter the method of detoxification used, it is hard to remove every trace of cyanide from the cassava tuber. The last trace of cyanide is detoxified by the body during digestion but as cyanide is broken down, thiocyanate; a goitrogenic compound is formed (McPhee *et al*., 2009). Thiocyanate can block thyroidal uptake of iodine by competing with sodium iodide symporter and this can consequently reduce uptake of iodide by the thyroid for thyroid hormone synthesis (McPhee *et al*., 2009).In areas where cassava is the staple food such as Ekiti East and Akoko Edo Local Government Areas in Ekiti and Edo states of Nigeria respectively, a high level of goitre (total goitre rate) has been observed (MediaGlobal, 2009).

The consumption of soybeans and its products has also been on the rise as a result of its high protein content (38-45%). It is used as a substitute for meat

(tofu) and dairy products (soymilk and soy cheese), oils, food seasonings and as an additive in diary milk (soy lecithin), processed cereal and infant formula. For a long time, there have been concerns on the subject of soybeans (*Glycine max*) effects on thyroid function and hormonal health. Many researchers have observed and reported that soybeans have isoflavones. McCarrison (1933) observed an elevated incidence of goitre in rats which consumed a diet that had raw soybeans which persisted even when the rats ingested large quantities of iodine. Sharpless *et al.* (1939) reported that soybean flour contains a goitrogenic agent which is moderately removed or destroyed by fat solvents (ether or acetone) or by steam. Wilgus *et al*. (1941) also found that the soybean agent was in some degree inactivated by heat. Sharpless *et al.* (1939) and Wilgus *et al.* (1941) showed that addition of minute quantities of iodine to the diets high in goitrogens will chiefly put a stop to the thyroid enlargement.

On the other hand, excess consumption of iodine inhibits three steps in the synthesis of thyroid hormones; iodide trapping, thyroglobulin iodination (wolf-Chaikoff effect) and thyroid hormone release from the thyroid gland (Wolf and Chaikoff, 1948) which can lead to hypothyroidism. Excessive iodine consumption can also induce hyperthyroidism and this is known as the Jod-Basedow effect. This occurs in patients with latent Graves' disease, multi-nodular thyroid and rarely in people with normal thyroid glands (Philips, 2012).

1.2 Justification

Previous investigations have shown that both hypothyroidism and hyperthyroidism caused dysfunction in glucose tolerance and led to increased fasting blood glucose in non-diabetic rats (Arigi *et al*., 2014). There is however, limited information on the effect of excessive iodine and goitrogenic foods combination on glucose homeostasis. Therefore, this study was designed to investigate the role of excess iodine and concurrent goitrogenic foods intake on thyroid function and glucose homeostasis in rats.

1.3 General Objectives

The general objectives of this study are to;

- Measure the effects of high iodine intake on glucose homeostasis, thyroid

function and oxidative stress.

- Determine the effects of diets high in goitrogenic foods (30% *Glycine max* and *Manihot esculenta)* on glucose homeostasis, thyroid function and oxidative stress.
- Assess the effects of concurrent intake of iodine and goitrogenic foods (30% *Glycine max* and *Manihot esculenta)* on glucose homeostasis, thyroid function and oxidative stress.
- Compare the effects of goitrogenic foods and/or iodine on glucose homeostasis, thyroid function and oxidative stress in non-diabetic and diabetic rats.

1.4 Specific objectives

The specific objectives of this research were to investigate the roles of excess iodine and goitrogenic foods on the following;

Thyroid function

Mean serum concentrations of triiodotyronine, thyroxine and thyroid stimulating hormones. Presence of thyroid antibodies (thyroperoxidase antibody and thyroglobulin antibody) and body weight changes.

Glucose Homeostasis

Mean fasting blood glucose level, oral glucose tolerance, insulin tolerance, fasting serum insulin concentrations and body weight changes.

Oxidative stress

Mean serum concentrations of nitric oxide, superoxide dismutase and malondialdehyde.

CHAPTER TWO

Literature Review

2.0 This chapter reviews literature in line with the introduction and research title

2.1 Iodine

Iodine is a non-metallic, naturally- occurring chemical element that is a member of the halogen family in Group VIIA of the periodic table. The chemical symbol for iodine is I. It occurs in nature as iodide (i.e., I^-) which is found in fish, seaweeds, shell-fish and seawater but exists as iodate or IO^{3-} in brines or in molecular compounds with other elements (Cooper, 2007). Isolation of elemental iodine (I_2) first occurred in the year 1811. Iodine has a dark purple colour at room temperature. It is a lustrous and crystalline solid. On heating it melts to form a liquid at 113.5^0C and boils to a pinkish purple vapour at 184.4^0C, but can sublime to vapour directly from the solid, depending on conditions. Its name is obtained from the Greek word 'iodes' for violet (Cooper, 2007).

Iodine has an atomic number of 53 and an atomic mass of 126.904. It is the least reactive halogen. Iodine dissolves readily in ethanol or ether to form brown solutions. It dissolves in chloroform or benzene to form violet solutions. It is sparingly soluble in water (0.33g/l, 1.2mM, at 25° C) giving a yellowish brown solution (Cooper, 2007).

Iodine solubility increases when iodide ions are present such as potassium iodide where iodine reacts to produce tri-iodide ions. Aqueous solutions of iodine are unstable and, depending on circumstances, lots of different varieties may be present. Of the different varieties of iodine, molecular iodine (I_2) is deemed to have the highest antimicrobial potential. Activity usually diminishes with longer storage time and higher alkalinity and stability is influenced by pH

(ATSDR, 2004).

There are non-radioactive and radioactive forms of iodine. Most radioactive iodine are synthetic and are used in medical examinations and to treat certain ailments. Most radioactive forms of iodine change very rapidly (seconds to days) to stable elements

that are not radioactive. However, 129I (read as iodine 129) changes very slowly (over millions of years). (ATSDR, 2004)Iodine is an essential micronutrient element. It is required for the formation of thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3), which are molecules of the amino acid tyrosine which have been iodinated (Haldiman *et al.*, 2014).

2.2 Food sources of iodine

The amount of iodine present in a particular food is determined by the iodine content of the soil in which that food is grown. A crop cultivated in a soil with higher iodine content will have a higher iodine content and food cultivated in a soil with less iodine content will have less iodine content (FAO/WHO, 2002).

Seawater is rich in iodine. Hence, vegetables such as seaweeds are a rich source of iodine. Fish that feed on seaweeds are also rich sources of iodine. Therefore, individuals that live close to the sea and consume sea-foods and reef fish such as some countries in parts of Asia have a high level of iodine intake. A large variety of foods contain iodine, they include dried fruits, dried vegetables, eggs, milk and milk products, cereals, grains, dried legumes, but the excellent sources are restricted to salt-water fishes and shellfish. The amount of iodine contained in foods varies with geographic locations, ranging broadly from 30 µg/100 g to 800 µg/100 g (FAO/WHO, 2002). Therefore, the mean content of iodine in foods from one locality cannot be unanimously used for estimating the iodine intake for a different group of people.

2.3 Salt Iodisation

'Universal salt iodisation (USI) is when all salts that are consumed by both humans and animals are iodized to the internationally agreed recommended levels' (WHO, 2001). In almost every country in which deficiency of iodine is present, the most effective way to eliminate iodine deficiency disorders has been well recognised to be through universal salt iodisation (USI). National salt iodization programmes are now functional globally, and have followed a regular pattern of evolution, which includes the following phases;

- Decision phase: the reason for this phase is to make room for decision on universal salt iodisation which should be supported by regulation and standards industry mobilisation and a set plan for implementation.

- Implementation phase: this involves making sure that the equipments and infrastructures for proper salt iodisation are in place.
- Consolidation phase: this involves ensuing that the program is sustainable which can be achieved by proper monitoring and evaluation of universal iodisation.

Iodine Deficiency Disorders (IDDs) can only be eliminated completely if all the control programmes strictly adhered to.

Salt iodisation is achieved by adding predetermined quantities of potassium iodate, either as a dry solid or an aqueous solution, at the point of manufacture. Due to its stability, Iodate is recommended in preference to iodide. Questions of vital significance to national health authorities and salt manufacturers has been raised in regards to the stability of iodine in salt as well as the level of iodisation of salt. The implications of the questions are for programme effectiveness, safety, and cost. The suitable levels of iodisation are determined by making an accurate estimate of the amount of iodine that will be lost between the time of iodisation and the time in which the salt will be consumed by the end users. Minimising moisture content in iodised salt is also very important in maintaining stability of added iodine.

WHO/UNICEF/ICCIDD recommends that, in typical circumstances, where:

- Iodine lost from salt is 20% from site of to the household,
- Another 20% is lost in the process of cooking before consumption, and
- Average intake of salt is 10 g per person per day,

The concentration of iodine in salt at the point of production should fall within the range of 20-40 mg of iodine per kg of salt (i.e., 20-40 ppm of iodine), this will provide 150 µg of iodine per person per day. The iodine should be added as potassium (or sodium) iodate. Under these circumstances median urinary iodine levels will vary from 100-200 µg/l

2.4 Absorption of iodine

Iodine absorption from diet occurs in all part of the gut. Before ingested iodine

is absorbed, it is first converted to iodide ion. But for iodine contained in synthetic thyroid hormones which are ingested as hormone replacement therapy, iodine is absorbed in the oxidised form. Absorbed iodine circulates as plasma inorganic iodide and is taken up by the thyroid gland for synthesis of thyroid hormones. The remainder in the circulation that is not absorbed by the thyroid gland is excreted by the kidneys. The amount of iodine in urine is a good measure of iodine intake (Food and Agriculture Organisation FAO, 1998).

2.5 Iodine requirements

The proposed daily iodine intake recommendations of 1996 by the World Health Organisation (WHO), United Nations Children's Fund and the International Council for Control of Iodine Deficiency Disorders (ICCIDD) is Infants (first 12 months) 90µg/day, children (1-6 years) 90µg/day, schoolchildren (7-12 years) 120µg/day, Adolescents and adults- 150µg per day, pregnant and lactating women -200µg/day. The human body has about 15-20 mg of iodine with 70-80% within the thyroid gland. The thyroid gland takes up iodine which is oxidised by thyroid peroxidise in the presence of hydrogen peroxide. The oxidized iodine is coupled with the tyrosine components of thyroglobulin and forms 3-monoiodotyrosine and 3,5-diidothyrosine. These combine to form triiodothyronine (T_3) and thyroxine (T_4) residues on the thyroglobulin. These thyroid hormones are attached to thyroglobulin and stored as the main component of the thyroid colloid. Proteolytic enzymes subsequently release T_3 and T_4, from thyroglobulin into the blood. When these hormones are utilized in various body cells, they are replaced by T_3 and T_4 from the bound pool. The liberated iodine may be reutilized by the thyroid gland, while the remainder iodine is excreted in the urine. Typically, urine contains more than 90% of all ingested iodine. Most of the remainder is excreted in faeces and a small amount may be lost in sweat (IOM, 2001).

2.6 Excess iodine intake

Excess consumption of iodine inhibits three steps in thyroid hormone production; iodide trapping, thyroglobulin iodination (the Wolff-Chaikoff effect) and thyroid hormone release from the thyroid gland which result in hypothyroidism (Wolff, 1969). The normal thyroid gland 'escapes' after ten to

fourteen days from these effects of excess iodine (Wolff, 1969). These effects of excess iodine consumption sometimes cause iodine-induced thyroid dysfunction especially if there is a previous underlying thyroid problem like autoimmune thyroiditis and hypothyroidism can develop (Braverman *et al.*, 1994). Excessive ingestion of iodine can also lead to hyperthyroidism (Jod-Basedow effect) in some patients with multi-nodular goitre, latent Grave's disease and rarely in individuals with a normal thyroid gland (Food and Agriculture Organisation FAO, 1998).

According to Leung and Braverman (2016), sources of iodine overconsumption include the following;

Iodine supplementation: Iodine supplementation has reduced the rate of iodine deficiency symptoms. It has also led to concerns of excessive iodine exposure in some individuals. Sang *et al.,* (2012) reported subclinical hypothyroidism in individuals supplemented with a 400µg iodine tablets than in those given placebo. Other studies by Laurberg *et al.*, (2006) and Thomson *et al.*, (2011) showed increased incidence of transient hyperthyroidism. Thyrotoxicosis was observed by Galofre *et al.*, (1994) and Todd *et al.*, (1995) following periods of mandatory salt iodisation.

Diet

Certain diets have high iodine content. These include dairy products, some breads, seaweed and other seafood. This high iodine content is mostly obtained from fertilizers, livestock feed, water used for irrigation and cleaning agents (Leung and Braverman, 2016).

Medications

Some medications rich in iodine such as amiodarone used in the management of ventricular and supraventricular tachyarrhythmias is likely the commonest and most important source of medication-induced thyroid dysfunction. It has been linked with thyrotoxicosis (Leung and Braverman, 2016).

Radiologic studies

The use of iodinated contrast agents in diagnostic radiological studies is a

common source of excess iodine exposure in many patients. A single dose of iodinated contrast contains up to 13,500µg of free iodine and 15-60µg of bound iodine(several thousand times above the RDI for iodine). (Rhee *et al.*, 2012)

Other sources

These include exposure to expectorants, food preservatives, parenteral nutrition preparations, mouthwashes, iodinated drinking water and vaginal douches. (Leung and Braverman, 2016).

Excess consumption of salt has never been documented to be responsible for excess iodine intake. Occasionally, each of these may have significant effects, but generally they are tolerated without difficulty. Braverman *et al.,* (1994) stated that people with no underlying thyroid disease almost always escape the acute inhibitory effects of excess intra-thyroidal iodide on the organification of iodide and subsequent hormone synthesis (escape from or adaptation to the acute Wolff-Chaikoff effect). High intakes of iodine from food, water and supplements have been associated with thyroidittis, goitre (due to increased thyroid stimulating hormones (TSH) stimulation, hypothyroidism, hyperthyroidism, sensitivity reactions, thyroid papillary cancer and acute responses in some individuals. Symptoms of acute iodine poisoning include burning of the mouth, throat, and stomach, abdominal pain, fever, nausea, vomiting, diarrhoea, weak pulse, cardiac irritability, coma and cyanosis (IOM, 2001).

2.7 Factors affecting the iodine requirement

Bioavailability

According to Albert and Keating, (1949), there is a higher than 90 percent rate of gastrointestinal absorption of iodine from the diets in typical conditions. When organic compounds of iodine are ingested, their rate of absorption is different from that of iodine. Oral administration of thyroxine provides a bioavailability of about 75 percent (Hays, 1991). Bioavailability may also be

affected by the presence of a substance that inhibits iodine absorption. It has been revealed that soybean flour inhibits the absorption of iodine in the digestive tract (Pinchera *et al.*, 1965). This must have led to the observation of hypothyroidism and goitre observed by (Shepard *et al.*, 1960) in infants who were fed on infant formula which contained soybean flour. (Shepard *et al.*, 1960) also observed that there was absence of goitre in infants when iodine was added to the formula.

Goitrogens

Goitrogens which interfere with the synthesis and utilisation of thyroid hormones are present in some foods (Gaitan, 1989). Examples of such foods include cassava, which may contain cyanide and is metabolized to thiocyanate which in turn can block thyroidal uptake of iodine; millet, soybeans (and soybean products such as tofu, soybean oil, soy flour, soy lecithin) which contain isoflavones, Pine nuts, peanuts, flax seed, millet, strawberries, pears, peaches, spinach, bamboo shoots, sweet potatoes, cruciferous vegetables such as broccoli, brussel sprouts, cabbage, canola, cauliflower, Chinese cabbage, collard greens, horseradish, kale, kohlrabi, mustard greens, radishes, rapeseed, rutabagas and turnips. Most of these substances are not of major clinical importance unless there is coexisting iodine deficiency. Deficiencies of vitamin A, selenium, or iron can each exacerbate the effects of iodine deficiency.

Other Factors

Many ingested substances contain large amounts of iodine that can interfere with proper thyroid function. These include radio contrast media, food colouring, certain medicines (e.g., amiodarone), water purification tablets, and skin and dental disinfectants.

Data suggest that the increased thyroid stimulating hormone levels found following erythrosine ingestion is related to anti-thyroid effects of increased

serum iodide concentrations, rather than a direct effect of erythrosine on thyroid hormones (Gardner *et al.*, 1987).

Similar to erythrosine, amiodarone, a highly effective anti-arrhythmic drug that contains high levels of iodine, may alter thyroid gland function (Loh, 2000). Radiographic contrast media, following intravascular administration, results in the formation of iodinated serum proteins, which alter thyroid metabolism (Nilsson *et al.*, 1987).

2.8 Iodine deficiency disorders

Hetzel introduced the term "iodine deficiency disorders" (IDD) in 1983. According to him, it included a large number of clinical manifestations, including foetal damage and loss, endemic cretinism, impaired mental function, as well as goitre. In Nigeria, Iodine Deficiency Disorders (IDDs) have been a public health problem for three decades. Data from 1988 found Total Goitre Rate (TGR) of 67% in endemic states which declined to 10.6% in 1998 (UNICEF, Nigeria 2004)

Symptoms of IDDs

According to Hetzel (1983) all the symptoms of iodine deficiency are associated with its effect on the thyroid. They are;

Goitre: Ingestion of inadequate amounts of iodine results in hypothyroidism which causes the anterior pituitary to increase the secretion of thyroid stimulating hormone. Thyroid stimulating hormone will stimulate the thyroid gland to begin to enlarge over time and this results in non-toxic or endemic goitre. Goitre can lead to choking especially when in a lying position. Goitre may also lead to difficulty in swallowing and breathing.

Hypothyroidism: Iodine deficiency can lead to hypothyroidism since iodine is essential in the synthesis of thyroid hormones. Globally, iodine deficiency is the most frequent root of hypothyroidism.

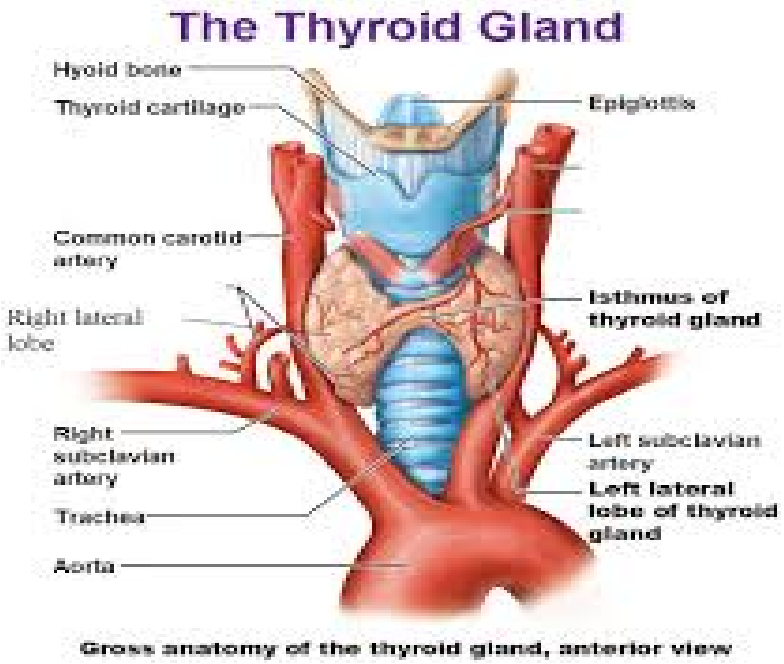
Pregnancy-related problems: Iodine deficiency in the diet of pregnant women and nursing mothers can lead to miscarriages, stillbirth, preterm delivery and congenital abnormalities in their babies. Because thyroid hormones are

essential for growth and the development of the central nervous system, children born to mothers with severe iodine deficiency during pregnancy can have cretinism which is characterised by mental retardation and problems with growth, hearing and speech. Congenital hypothyroidism due to iodine deficiency is the most preventable cause of mental retardation in the world. Even mild iodine deficiency during pregnancy may be associated with low intelligence quotient in children.

2.9 The thyroid gland

The thyroid gland is the largest gland in the neck below the thyroid cartilage. It is located in the anterior part of the neck under the skin and muscle layers. It has a butterfly shape with the two wings representing the left and right thyroid lobes which wrap around the trachea. The main role of the thyroid gland is the synthesis of thyroid hormones (Norman 2012).

Fig 2.1. Diagram of the thyroid gland



Antranik.org

Surrounding the thyroid gland is a fibrous sheath known as the capsula glandulae thyroidea which is made up of both and internal and external layer. The external layer is continous anteriorly with the lamina pretrachealis fascia cervicalis and continuous posterior-laterally with the carotid sheath. Anteriorly,

the thyroid gland is surrounded by infrahyoid muscles and laterally by the sternocleidomastoid muscle also known as the sternomastoid muscle. Posteriorly, the thyroid gland is fixed to the cricoid and tracheal cartilage and cricopharyngeus muscle by a thickening of the fascia to form the posterior suspensory ligament of Berry (Yalkin and Ozan, 2006) (Lemaire and David, 2005). The firm attachment of the thyroid gland to the underlying trachea is the reason why it moves during deglutition (Venturi *et al.*, 2000). In variable extent, Laloutte's Pyramid, a pyramidal extension of the thyroid lobe, is present at the most anterior side of the lobe. In this region, then recurrent laryngeal nerve and the inferior thyroid artery pass next to or in the ligament and tubercle.

Two parathyroid glands are located between the two layers of the capsule and on the posterior side of the lobes.

The thyroid is one of the larger endocrine glands, weighing 2-3 grams in neonates and 18-60 grams in adults, and is increased in pregnancy.

Blood supply

The blood supply to the thyroid is through the superior thyroid artery, the inferior thyroid artery and sometimes the thyroid ima artery. The superior thyroid artery branches from the external carotid artery, the inferior thyroid artery is a branch of the thyrocervical trunk and the thyroid ima artery branches directly from the brachiocephalic trunk. The venous blood is drained through the superior thyroid veins, draining in the internal jugular vein, and via inferior thyroid veins, draining via the plexus thyroideus impar in the left brachiocephalic vein. Relative to its size, the thyroid receives a greater flow of blood than other parts of the body (Leonard 2003)

Lymphatic drainage passes frequently through the lateral deep cervical lymph nodes and the pre-and para-tracheal lymph nodes. The gland is supplied by parasympathetic nerve input from the superior laryngeal nerve and the recurrent laryngeal nerve.

Histology

The follicle of the thyroid gland is its functional unit. The follicle is made up of

epithelila cells arranged as hollow vessicles of various shapes ranging in size from 0.02-0.03mm in diametre; it is filled with a glycoprotein colloid called thyroglobulin. The adult human thyroid gland has about three million follicles. Epithelial cells lining each follicle may be cuboidal or columnar depending on their functional state, with the height of the epithelium being greater when it's activity is highest. Each follicle is surrounded by a dense capillary network separated from epithelial cells by a well defined basement membrane. Groups of densely packed follicles are bound together by connective tissue to form lobules that receive their blood supply from a single small artery. The functional state of one lobule may differ widely from that of an adjacent lobule.

2.10 Synthesis of thyroid hormones

The biosynthesis of thyroid hormones involves a series of processes which are dependent on the product of three genes that are expressed predominantly, if not exclusively, in thyroid follicle cells: the sodium iodide symporter (NIS), thyroglobulin, and thyroid peroxidase (Leonard 2003)

Iodide Pump (Iodide Trapping)

The first step in thyroid hormone synthesis is the transport of iodides from the blood into the thyroid glandular cells and follicles. This process is known as iodide trapping (Leonard, 2003). Under normal circumstances, the sodium-iodide symporter can concentrate iodide to about 25–50 times more in the cytosol of thyroid follicular cells than in blood plasma, and during periods of active stimulation, it may be as high as 250 times that of plasma. Iodine is accumulated against a steep concentration gradient by the action of an electrogenic ‘‘iodide pump’’ located in the basolateral membranes (Moit *et al.*, 2010). In a normal gland, the iodide pump concentrates the iodide to about 30 times its concentration in the blood (Leonard, 2003).

The pump couples the transfer of two ions of sodium with each ion of iodide. The sodium/iodide symporter or NIS is located at the basolateral plasma membrane of thyrocytes. NIS belongs to the sodium/glucose co-transport

family as the SLC5A5 member (Moit *et al.*, 2010). Iodide is thus transported against its concentration driven by the favourable electrochemical gradient for sodium. Like other transporters, the sodium iodide symporter has a finite capacity and can be saturated. Consequently, other anions, e.g., perchlorate, pertechnetate, and thiocyanate that compete for binding sites on the sodium iodide symporter can block the uptake of iodide. This property can be exploited for diagnostic or therapeutic purposes.

Iodide supply of follicular lumen involves a two-step transport process: the active transport across the basolateral plasma membrane of thyrocytes by NIS and a passive transport across the apical plasma membrane.

The rate of iodide trapping by the thyroid is influenced by several factors, the most important being the concentration of TSH; TSH stimulates and hypophysectomy greatly diminishes the activity of the iodide pump in thyroid cells (Guyton and Hall 2006). Once inside follicular cells, the iodide ions diffuse rapidly to the apical membrane, where they are used for iodination of the thyroglobulin precursor (Rhoades and Tanner 2004).

Most dietary iodine is reduced to iodide before absorption throughout the gut, principally in the small intestine. Absorption is virtually complete. Iodinated amino acids, including T_4 and T_3, are transported intact across the intestinal wall. Short-chain iodopeptides may also be absorbed without cleavage of peptide bonds (Wynn, 1961). Iodinated dyes used in radiography are absorbed intact, but some deiodination occurs later. Except in the postabsorptive state, the concentration of iodide in the plasma is usually less than 10 µg/L. Absorbed iodide has a volume of distribution numerically equal to about 38% of body weight (in kilograms) (DeGroot, 1966), mostly extracellular, but small amounts are found in red cells and bones.

The thyroid and kidneys remove most iodide from the plasma. The renal clearance of iodide is 30-50 ml plasma/min (DeGroot, 1966) and appears largely independent of the load of iodide or other anions. In certain species, such as the rat, large chloride loads can depress iodide clearance. In humans, renal iodide clearance depends principally on glomerular filtration, without evidence of tubular secretion or of active transport with a transfer maximum

(Perry and Hughes 1952). Reabsorption is partial, passive, and depressed by an extreme osmotic diuresis. Hypothyroidism may decrease and hyperthyroidism may increase renal iodide clearance, but the changes are not marked (DeGroot, 1966).

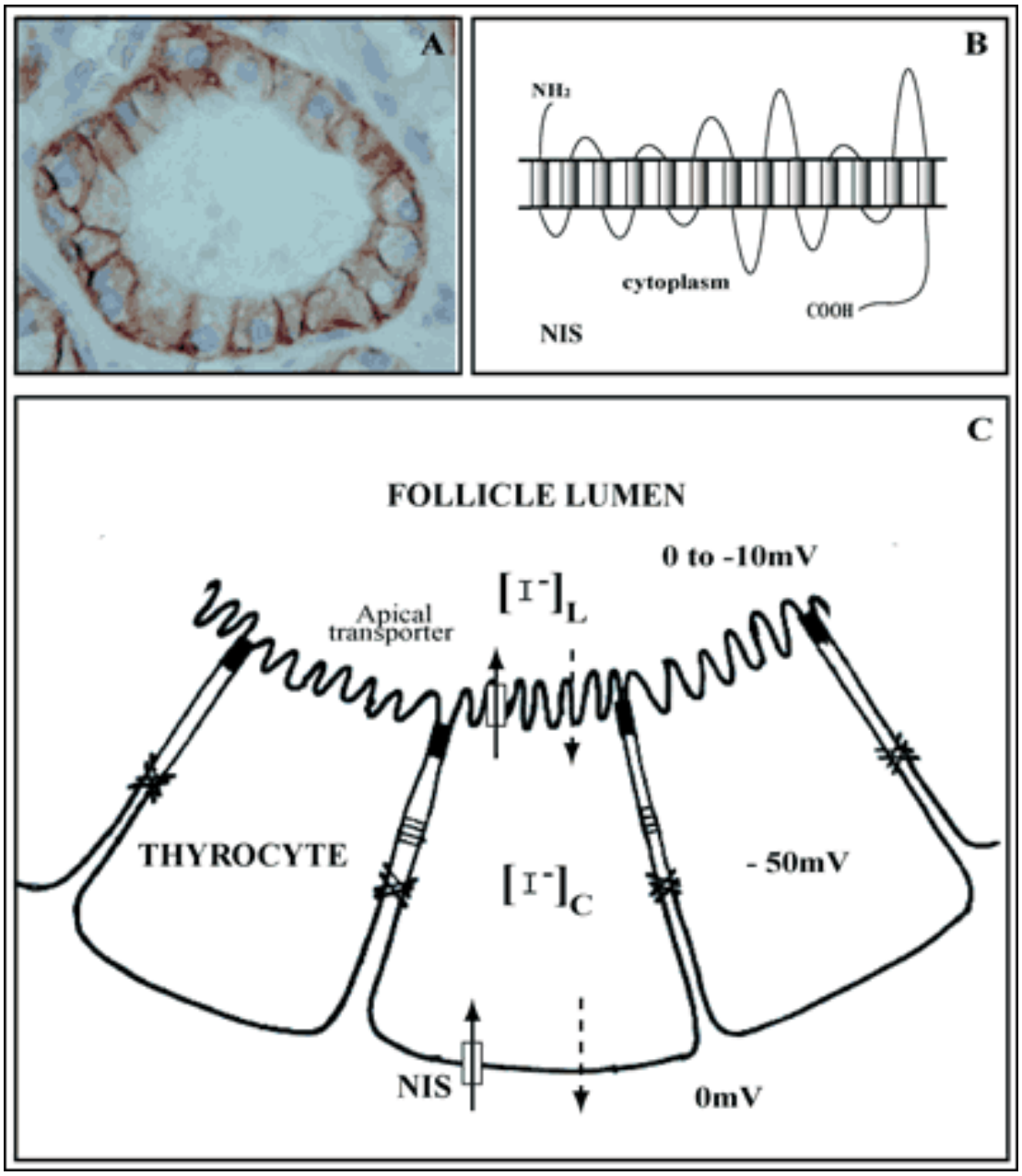


Fig 2.2.NIS-mediated transport of iodide(Moit *et al.*, 2010)

A: Immunolocalization of the human NIS protein at the basolateral plasma membrane of thyrocytes in their typical follicle organization.

B: Schematic representation of the membrane topology of the NIS polypeptide

chain deduced from secondary structure prediction analyses.

C: Transport of iodide from the extracellular fluid (or plasma) to the thyroid follicle lumen. The uptake of iodide at the basolateral plasma membrane of thyrocytes must be active; it operates against an electrical gradient (0 - 50 mV) and a concentration gradient. (Moit *et al.*, 2010)

Thyroperoxidase (TPO)

After concentrating iodide, the thyroid rapidly oxidizes it and binds it to tyrosyl residues in Thyroglobulin, followed by coupling of iodotyrosines to form T_4 and T_3. The process requires the presence of iodide, a peroxidase, a supply of H_2O_2, and an iodine acceptor protein (Tg).

Thyroperoxidase (TPO) oxidizes iodide in the presence of H_2O_2. It can be solubilized using detergents such as deoxycholate or digitonin. The enzyme activity is dependent on the association with a heam, the ferriprotoporphyrin IX or a closely related porphyrin (Czarnocka *et al.*, 1985). Chemical removal of the prosthetic group inactivates the enzyme, and recombination with the heam protein restores activity (Czarnocka *et al.*, 1985). The apoprotein from human thyroid is not always fully saturated with its prosthetic group (Kortani *et al.*, 1986). Some congenitally goitrous children have poor peroxidase function because the apoprotein has weak binding for the heam group (Kortani *et al.*, 1986).

Antibodies directed against the thyroid “microsomal antigen,” which are present in the serum of patients with autoimmune thyroid disease (AITD), led to identification of TPO. These antibodies were found to react with proteins of 101-107 kDa and to immunoprecipitate thyroid peroxidase (TPO), thus identifying microsomal antigen as TPO (Ruff *et al.*, 1987) (Kimura *et al.*, 1987) (Hamara *et al.*, 1987).

Thyroglobulin (Tg)

Thyroglobulin is the other major component needed synthesis of thyroxine and triiodothyronine. Thyroglobulin is the most abundant protein in the thyroid gland; its concentration within the follicular lumen can reach 200-300 g/L

(Leonard, 2003). Its main function is to provide the polypeptide backbone for synthesis and storage of thyroid hormones (Alvino *et al.*, 1995). It also offers a convenient depot for iodine storage and retrieval when external iodine availability is scarce or uneven. It is a large glycoprotein that forms a stable dimer with a molecular mass of about 660,000 Da. Like other secretory proteins, thyroglobulin is synthesized on ribosomes, glycosylated in the cisternae of the endoplasmic reticulum, translocated to the Golgi apparatus, and

packaged in secretory vesicles that discharge it from the apical surface into the lumen. Because thyroglobulin secretion into the lumen is coupled with its synthesis, follicular cells do not have the extensive accumulation of secretory granules that is characteristic of protein secreting cells. Iodination to form mature thyroglobulin does not take place until after the thyroglobulin is discharged into the lumen (Leonard 2003).

Tg contains sulphur and phosphorus. The former is present in the chondroitin sulfate and the complex carbohydrate units, although its form and role are not known (Gartner *et al.*, 1996). Several studies have reported phosphate in Tg, up to 12 mol. per mol Tg. Of this, about half is in the complex carbohydrate units, the remainder is present as phosphoserine and phosphotyrosine (Peirera *et al.*, 1990) (Paneels *et al.*, 1996). This may relate to protein kinase A activity (Paneels *et al.*, 1994).

Formation of the iodothyronine residues.

The next step in the formation of thyroglobulin is the addition of one or two iodine atoms to certain tyrosine residues in the precursor protein (Leonard 2003). The precursor of thyroglobulin contains 134 tyrosine residues, but only a small fraction of these become iodinated. A typical thyroglobulin molecule contains only 20 to 30 atoms of iodine. The iodination of thyroglobulin is catalyzed by the enzyme thyroid peroxidase, which is bound to the apical membranes of follicular cells. Thyroperoxidase oxidizes iodide in the presence of H_2O_2(Leonard 2003). In crude thyroid homogenates, enzyme activity is associated to cell membranes. By definition, a peroxidase requires H_2O_2 for its oxidative function.Thyroid peroxidase binds an iodide ion and a tyrosine

residue in the thyroglobulin precursor, bringing them in close proximity.

One scheme proposes that oxidation produces free radicals of iodine and tyrosine, while both are bound to TPO to form monoiodothyronine (MIT) which then separates from the enzyme. Further reaction between free radicals of iodine and MIT gives diiodothyronine (DIT)(Paneels *et al.*, 1994).

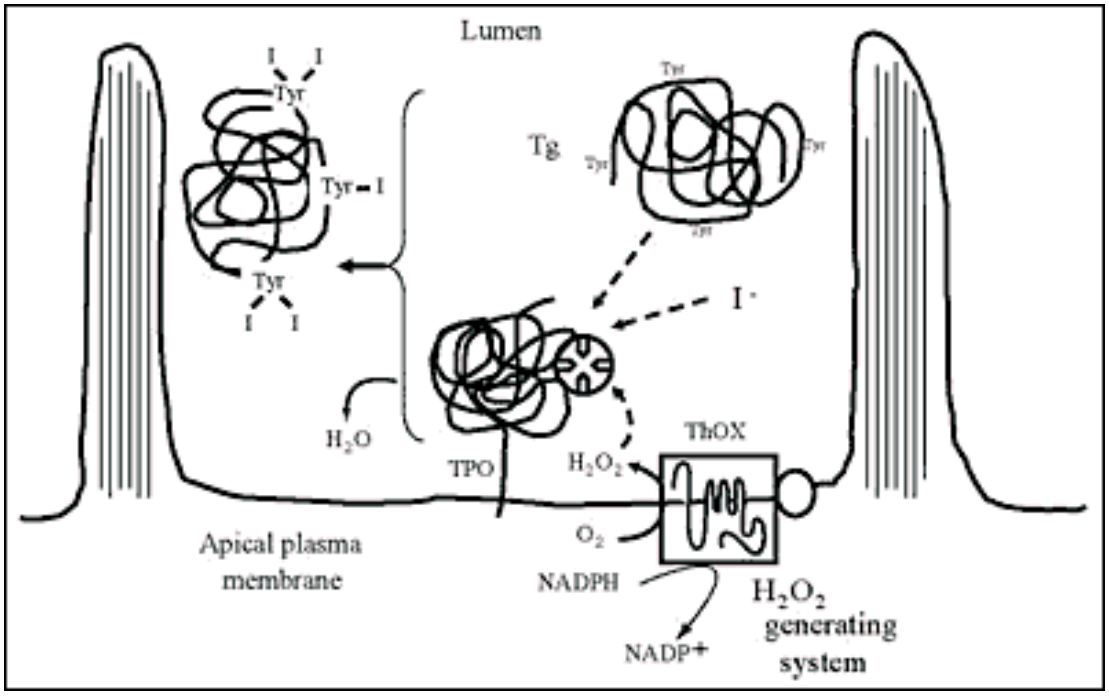


Fig. 2.3. Figure illustrating the Iodination of Tg at the apical plasma membrane-follicle lumen boundary.The scheme does not account for the relative size of the intervening molecules. (Moit *et al.*, 2010)

The final step in hormone synthesis is the coupling of two neighboring iodotyrosyl residues to form iodothyronine. Two DIT form T_4; one DIT and one MIT form T_3. Coupling takes place while both acceptor and donor iodotyrosyl are in peptide linkage within the Tg molecule.The reaction is catalyzed by TPO, requires H_2O_2 (Ekholm, 1981) (Ofverholm and Erricson, 1984) (Herzog *et al.*, 1992) and is stringently dependent on Tg structure (Berndorfer *et al.,* 1996).The generation of the iodothyronine residue involves the formation of an ether bond between the iodophenol part of a donor tyrosyl and the hydroxyl

group of the acceptor tyrosyl. After the cleavage reaction that gives the iodophenol, the alanine side chain of the donor tyrosyl remains in the Tg polypeptide chain as dehydroalanine (Baundry *etal.*, 1998). Observations both in vivo and in vitro show an appreciable delay in coupling after initial formation of iodotyrosines. A typical distribution for a Tg containing 0.5% iodine (a normal amount for iodine-sufficient individuals) is 5 residues MIT, 5 of DIT, 2.5 of T_4 and 0.7 of T_3 (Alvino *et al.*, 1995). More iodine increases the ratios of DIT/MIT and T_4/T_3, while iodine deficiency decreases them.

Only about 20 to 25% of the DIT and MIT residues in the thyroglobulin molecule become coupled to form iodothyronines. For example, a typical thyroglobulin molecule contains five to six uncoupled residues of DIT and two to three residues of T_4. However, T_3 is formed in only about one of three thyroglobulin molecules. As a result, the thyroid secretes substantially more T_4 than T_3 (Rhoades and Tanner 2007).

2.11 Thyroid hormone storage

Thyroglobulin molecules vectorially delivered to the follicule lumen by exocytosis accumulates to reach uncommon concentrations i.e. 0.3-0.5 mM. The mechanism operating such a “packaging” is unknown. Water and ion extraction from the follicle lumen might represent an active process leading to Tg concentration (Moit *et al.*, 2010). The high degree of compaction of luminal Tg might depend on electrostatic interactions between Ca^{2+} and anionic residues of Tg, which is an acidic protein. Stored Tg molecules undergo iodination and hormone formation reactions at the apical plasma membrane-lumen boundary (Dunn *et al.*, 1982, 1988), where TPO and H_2O_2 generating system reside. The mature Tg molecules, now containing MIT, DIT, T_4 and T_3, remains extracellular in the lumen of thyroid follicles. Turnover of intra-follicular material or so-called colloid varies greatly with gland activity. For normal humans, the organic iodine pool (largely in intra-follicular material), turns over at a rate of about 1% per day. When the turnover increases, less Tg is stored, and with extreme hyperplasia, none is evident and the entire organic iodine content may be renewed daily. In this situation, secretion of Tg and resorption of Tg probably occur at similar rates and only tiny amounts of intra-follicular material are present at any time (Moit *et al.*, 2010).

2.12 Secretion of thyroid hormones

Thyroid hormones are released form thyroglobulin and released to the circulation in order to be able to carry out their actions in the target tissues. The Tg hormone content of the luminal Tg molecules vary to a large extent depending on numerous factors including – the supply of iodide as substrate, the activity of enzymes catalyzing hormone formation, the concentration and physico-chemical state of Tg. The downstream processes responsible for the production of free thyroid hormones from these prohormonal molecules must therefore adequately manage the use of these luminal heterogeneous Tg stores to provide appropriate amounts of hormones for peripheral utilization. Therefore, the following will be expected

i. Control systems preventing excess hormone production that would result from the processing of excessive amounts of prohormonal Tg molecules and

ii. Checking systems avoiding the use of Tg molecules with no or a low hormone content.

2.13 Chemistry of thyroid hormones

Thyroid hormones are derivatives of the amino acid tyrosine bound covalently to iodine. The two principal thyroid hormones are:

Thyroxine (also known as T_4 or L-3,5,3',5'-tetraiodothyronine)

Triiodothyronine (T_3 or L-3,5,3'-triiodothyronine)

Although the main secretory product of the thyroid gland and the major form of thyroid hormone present in the circulating plasma reservoir is T_4, abundant evidence indicates that it is T_3 and not T_4 that binds to the thyroid hormone receptor In fact, T_4 can be considered to be a pro-hormone that serves as the precursor for extra-thyroidal formation of T_3 (Leonard, 2003).

As shown in the diagram below, the thyroid hormones are basically two tyrosines linked together with the critical addition of iodine at three or four

positions on the aromatic rings (Bowen, 1999). The number and position of the iodines is important. Several other iodinated molecules are generated that have little or no biological activity; so called "reverse T_3" (3,3',5'-T_3) is such an example..

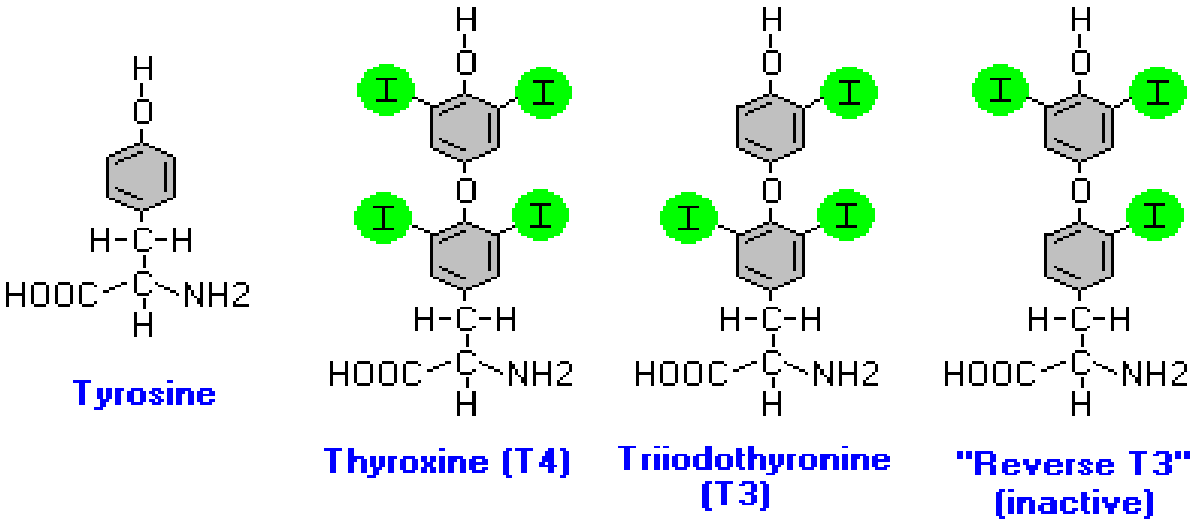


Fig.2.4: Structure of thyroid hormones

Courtesy: Bowen (1999)

Thyroid hormones are poorly soluble in water, and more than 99% of the T_3 and T_4 circulating in blood is bound to carrier proteins. The principle carrier of thyroid hormones is thyroxine-binding globulin, a glycoprotein synthesized in the liver. Two other carriers of import are transthyretin and albumin. Carrier proteins allow maintenance of a stable pool of thyroid hormones from which the active, free hormones are released for uptake by target cell.

2.14 Control of thyroid hormone synthesis and secretion

The most important controlling factors are iodine availability and TSH. Inadequate amounts of iodine lead to inadequate thyroid hormone production, increased TSH secretion and thyroid stimulation, and goitre in an attempt to compensate. A proposed mechanism is that the excess iodide leads to the formation of 2-iodohexadecanal (Johanson *et al.*, 1988), which is endowed with an inhibitory action on H_2O_2 generation.

TSH influences virtually every step in thyroid hormone synthesis and release.

In humans the effects on secretion appear to be mediated through the cAMP cascade while the effects on synthesis are mediated by the Gq/phospholipase C cascade (Song *et al.*, 2010).

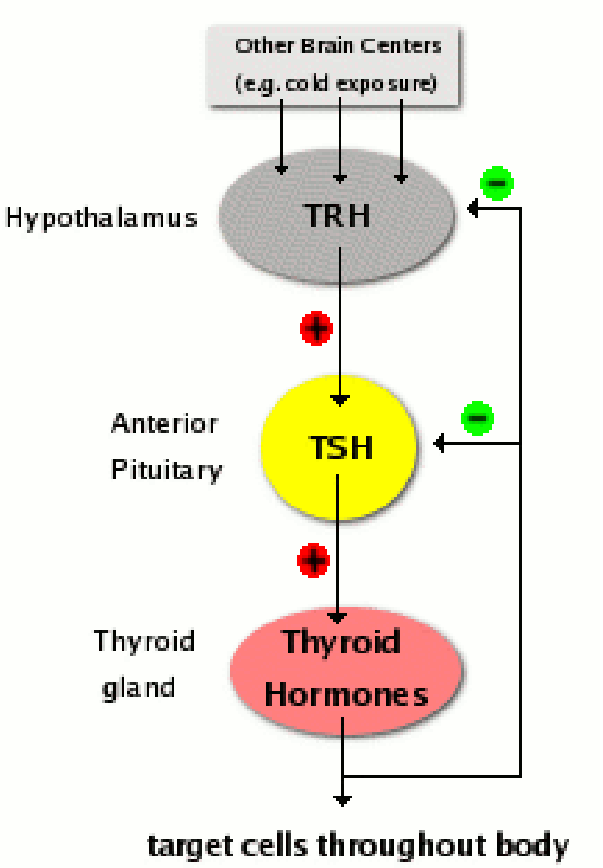


Fig. 2.5.Control of thyroid hormone synthesis and secretion.
Diagram courtesy: Bowen (1999)

Excess iodide acutely inhibits thyroid hormone synthesis, the Wolff-Chaikoff effect (Consiglio, 1979), apparently by inhibiting H_2O_2 generation, and therefore, blocking Tg iodination (Consiglio, 1979).

2.15 Thyroid Stimulating Hormone

The pituitary-thyroid axis has served neuro-endocrinology as the example par excellence of a negative-feedback self-regulatory system. This regulation is achieved by the interaction of three groups of hormones: hypothalamic, pituitary and thyroid. Hypothalamic hormones are thyrotropin-releasing hormone (TRH), which stimulates the synthesis and release of thyrotopin (Thyroid Stimulating Hormone: TSH), somatostatin, which inhibits TSH secretion and dopamine, which is also inhibitory. Thyroid stimulating hormone in turn activitates iodide uptake, homonogenesis and release of the thyroid hormones; thyroxine (T_4) and triidothyronine (T_3). The circulating thyroid

hormones exert negative feedback effects on the pituitary to regulate TSH secretion. In addition, metabolic conversion of T_4 to the more active T_3 takes place in both the pituitary gland and the hypothalamus.

Although this model describes most of the regulatory factors in pituitary-thyroid function, other mechanisms influence the rate of TSH secretion. These include the physical state of thyroid hormones in the blood and the peripheral degradation of TSH, of thyroid hormones and of TRH.

Chemistry

Human pituitary TSH is a glycoprotein of molecular weight 28,000 Daltons secreted by a specific type of basophilic cells of the anterior pituitary gland (thyrotrope cells). About 5 percent of adenohypopysial cells are thyrotropes.

TSH consists of two chemical subunits, alpha and beta. The alpha subunit is devoid of biologic activity. The beta subunit determines the biologic and immunologic activity of TSH.

TSH stimulation hormone synthesis and release are stimulated by TSH and inhibited by thyroid hormones. Normal serum levels in man are less than 8μU/ml. thyroid stimulating hormone circulates unbound in the blood and has a half-life of 50 to 60 minutes. Approximately 10 to 30 percent of total pituitary TSH content is secreted daily.

Secretory pattern

TSH is secreted in pulses highest levels are usually observed during the morning hours from four to eight am. Serum TSH levels rise transiently in the infant immediately after birth, a peak occurring within the first half of life. Thyroid stimulating hormone levels return to baseline adult values within 48 to 72 hours.

2.16 Transport of thyroid hormone

More than 99% of thyroid hormone circulating in blood is firmly bound to three plasma proteins. They are thyroxine-binding globulin (TBG), transthyretin (TTR), and albumin (Rhoades and Tanner, 2003). About 70% of the T_4 and 80% of the T_3 are non-covalently bound to thyroxine-binding globulin (TBG), a 54-kDa

glycoprotein that is synthesized and secreted by the liver. Each molecule of TBG has a single binding site for a thyroid hormone molecule. The remaining T_4 and T_3 in the blood are bound to transthyretin or to albumin (Leonard, 2005). Less than 1% of the T_4 and T_3 in blood is in the free form, and it is in equilibrium with the large protein bound fraction. It is this small amount of free thyroid hormone that interacts with target cells. The protein-bound form of T_4 and T_3 represents a large reservoir of preformed hormone that can replenish the small amount of circulating free hormone. The type II deiodinase is absent from the liver, but is present in skeletal muscle, the CNS, the pituitary gland, and the placenta. Type 2 deiodinase is believed to function primarily to maintain intracellular T_3 in target tissues, but it may also contribute to the generation of circulating T_3 (Rhoades and Tanner, 2003). Expression of the type II deiodinase is regulated by other hormones; its expression is highest when blood concentrations of T_4 are low. In addition, hormones that act through the cyclic AMP second messenger system and growth factors stimulate type II deiodinase expression. These characteristics support the idea that this enzyme may provide T_3 to meet local demands.

The type III deiodinase removes an iodine from the tyrosyl ring of T_4 or T_3, and hence its function is solely degradative. It is widely expressed by many tissues throughout the body. Reverse T_3, a product of both the type I and type III deiodinases, may be further deiodinated by the type III deiodinase by removal of the second iodide from inner ring. Reverse T_3 is also a favoured substrate for the type I deiodinase, and although it is formed at a similar rate as T_3 it is degraded much faster than T_3. Some rT_3 escapes into the bloodstream where it is avidly bound to TBG and TTR.

All three thyroid hormone binding proteins bind T_4 at least 10 times more avidly than T_3. All are large enough to escape filtration by the renal glomerular membranes and little crosses the capillary endothelium. The less than 1% of hormone present in free solution is in equilibrium with bound hormone and is the only hormone that can escape from capillaries to produce biological activity or be acted on by tissue enzymes. The total amount of thyroid hormone bound to plasma proteins represents about three times as much hormone as is secreted and degraded in the course of a single day. Thus

plasma proteins provide a substantial reservoir of extra-thyroidal hormone. We should therefore not expect acute increases or decreases in the rate of secretion of thyroid hormones to bring about large or rapid changes in circulating concentrations of thyroid hormones (Leonard, 2005). For example, if the rate of thyroxine secretion were doubled for 1 day, we could expect its concentration in blood to increase by no more than 30% even if there were no accompanying increase in the rate of hormone degradation. A 10-fold increase in the rate of secretion lasting for 60 min would only give a 12% increase in total circulating thyroxine, and if thyroxine secretion stopped completely for 1 hr, its concentration would decrease by only 1%. Furthermore, because the binding capacity of plasma proteins for thyroid hormones is far from saturated, even a massive increase in secretion rate would have little effect on the percentage of hormone that is unbound. These considerations seem to rule out changes in thyroid hormone secretion as effectors of minute-to-minute regulation of any homeostatic process. On the other hand, because so much of the circulating hormone is bound to plasma binding proteins, we might expect that the total amount of T_4 and T_3 in the circulation would be affected significantly by decreases in the concentration of plasma binding proteins, as might occur with liver or kidney disease.

2.17 Metabolism of thyroid hormones

Whereas the 5'-deiodination of T_4 to produce T_3 can be viewed as a metabolic activation process, both T_4 and T_3 undergo enzymatic deiodinations, particularly in the liver and kidneys, which inactivate them (Rhoades and Tanner, 2003). For example, about 40% of the T_4 secreted by the human thyroid gland is deiodinated at the 5' position on the thyronine ring structure by a 5'-deiodinase. This produces reverse T_3. Since reverse T_3 has little or no thyroid hormone activity, this deiodination reaction is a major pathway for the metabolic inactivation or disposal of T_4. Triiodothyronine and reverse T_3 also undergo deiodination to yield 3,3'-diiodothyronine. This inactivate metabolite may be further deiodinated before being excreted.

Regulation of 5'-Deiodination

The 5'-deiodination reaction is a regulated process influenced by certain

physiological and pathological factors. The result is a change in the relative amounts of T_3 and reverse T_3 produced from T_4. For example, a human foetus produces less T_3 from T_4 than a child or adult because the 5'-deiodination reaction is less active in the foetus. Also, 5'-deiodination is inhibited during fasting, particularly in response to carbohydrate restriction, but it can be restored to normal when the individual is fed again. Trauma, as well as most acute and chronic illnesses, also suppresses the 5'-deiodination reaction. Under all of these circumstances, the amount of T_3 produced from T_4 is reduced and its blood concentration falls. However, the amount of reverse T_3 rises in the circulation, mainly because its conversion to 3,3'-diiodothyronine by 5'-deiodination is reduced. A rise in reverse T_3 in the blood may signal that the 5'-deiodination reaction is suppressed. Note that during fasting or in the disease states mentioned above, the secretion of T_4 is usually not increased, despite the decrease of T_3 in the circulation. This response indicates that, under these circumstances, a T_3 decrease in the blood does not stimulate the hypothalamic-pituitary-thyroid axis (Leonard, 2005).

2.18 Mechanisms of thyroid hormones actions

Thyroid hormones are quite hydrophobic and may either diffuse across the cell membrane or enter target cells by a carrier-mediated transport process. Receptors for thyroid hormones are intracellular DNA-binding proteins that function as hormone-responsive transcription factors, very similar conceptually to the receptors for steroid hormones (Bowen, 1999).

Thyroid hormones enter cells through membrane transporter proteins. T_3 formed within the target cell by deiodination of T_4 appears to mix freely with T_3 taken up from the plasma and to enter the nucleus, where it binds to specific receptors. Thyroid hormone receptors are members of the large family of nuclear hormone receptors and bind to specific nucleotide sequences (thyroid response elements or TREs) in the genes they regulate (Leonard, 2005). Unlike most other nuclear receptors, thyroid hormone receptors bind to their response elements in the absence of hormone. They bind as monomers or as homodimers composed either of two thyroid hormone receptors, or they may form heterodimers with other nuclear receptor family members, usually the receptor for an isomer of retinoic acid. In the absence of T_3, the unoccupied

receptor, in conjunction with a corepressor protein, inhibits T_3-dependent gene expression by maintaining the DNA in a tightly coiled configuration that bars access of transcription activators or RNA polymerase. Upon binding T_3, the configuration of the receptor is modified in a way that causes it to release the co-repressor and bind instead to a co-activator. Although T_3 acts in an analogous way to suppress expression of some genes, the underlying mechanism for negative control of gene expression is not understood.

TRs have been shown to belong to a large super-family of nuclear hormone receptors that include the steroid, vitamin D, and retinoic acid receptors as well as "orphan" receptors for which there are no known ligand or function (Yen, 2001).. It is highly likely that T_3 and T_4 have physiologically important actions that are not dependent on nuclear events, but detailed understanding will require further research (Leonard, 2005).

2.19 Effects on thyroid hormones on body systems.

Thyroid hormone receptors are expressed in virtually all tissues, although the relative expression of TR isoforms may vary among tissues. TRβ-1 mRNA is highly expressed in liver but also expressed in almost all other tissues, whereas TRβ-2 mRNA is most highly expressed in the anterior pituitary. TRα-1 is expressed in almost all tissues. In addition to this variable expression of TR isoforms in different tissues, the role of TH can vary in different tissues. Thus, in addition to its role on the metabolism of macronutrients and overall energy and oxygen consumption, TH also regulates important functions in specific tissues (Yen, 2001). The transcriptional regulation of target genes in some of these tissues has been studied.

2.20 Effects of thyroid hormones on the nervous system

Thyroid hormone has major effects on the developing brain in-utero and during the neonatal period (Bhat *et al.*, 1994) (Oppenheimer *et al.*, 1972). Neonatal hypothyroidism due to genetic causes and iodine deficiency in humans can cause mental retardation and neurological defects. Studies in hypothyroid neonatal rats have shown that absence of TH causes diminished axonal growth and dendritic arborization in the cerebral cortex, visual and auditory cortex, hippocampus, and cerebellum (Rabello *et al.*, 1995) (Rabie *et al.*, 1977).

In the cerebellum, absence of TH also delays proliferation and migration of granule cells from the external to the internal granular layer. The critical role of TH is further demonstrated by a report in which a dominant negative TR was targeted to the cerebellum in transgenic mice. The Purkinje cells showed decreased dendritic arborization, while the granule cells had retarded migration to the internal granular layer (Kiobuchi and Chin, 1998). The developmental delays in the rat brain can be reversed if TH is administered within 2 weeks after birth (Valcavi *et al.*, 1993). These findings support the clinical observations that early T_4 treatment of congenital hypothyroidism prevents intellectual impairment in humans and is the major impetus for neonatal screening for congenital hypothyroidism. In utero, monodeiodination of T_4 to T_3 by type II deiodinase and maternal-foetal transfer of T_4 may help maintain normal T_3 concentrations even when the foetus has congenital hypothyroidism (Davidson *et al.*, 1990). Additionally, maternal transfer of thyroxine may be important, particularly during early foetal development (Carre *et al.*, 1998). Recent studies have suggested that maternal thyroid status may have significant effects on the neuropsychological outcome of children (Benall, 1999)

The ontogeny of TR isoforms in the brain suggests that specific TR isoforms may be involved in transcription of target genes and in brain development (Sterling *et al.*, 1978). TRα-1 is expressed throughout the brain from early foetal development and accounts for total T_3 binding in the foetal brain. TRβ-1 is absent or minimally expressed, except in a few selected areas such as the cochlea and cerebellum (Blanco *et al.*, 1998) However, there is a dramatic 40-fold increase in TRβ-1 mRNA expression throughout the brain shortly after birth that reaches maximum levels 10 days afterward, and then persists until adulthood (Sterling *et al.*, 1978).

2.21 Effects of thyroid hormones on growth

Thyroid hormone has both general and specific effects on growth. For instance, it has long been known that thyroid hormone is essential for the metamorphic change of the tadpole into the frog. In humans, the effect of thyroid hormone on growth is manifest mainly in growing children. In those who are hypothyroid,

the rate of growth is greatly retarded. In those who are hyperthyroid, excessive skeletal growth often occurs, causing the child to become considerably taller at an earlier age. However, the bones also mature more rapidly and the epiphyses close at an early age, so that the duration of growth and the eventual height of the adult may actually be shortened. An important effect of thyroid hormone is to promote growth and development of the brain during foetal life and for the first few years of postnatal life. If the foetus does not secrete sufficient quantities of thyroid hormone, growth and maturation of the brain both before birth and afterward are greatly retarded, and the brain remains smaller than normal. Without specific thyroid therapy within days or weeks after birth, the child without a thyroid gland will remain mentally deficient throughout life (Guyton and Hall, 2006).

Thyroid Hormone regulates the synthesis and secretion of several pituitary hormones. Absence of GH has been observed in the pituitaries of hypothyroid rats. Co-transfection studies using the human growth hormone promoter have not shown stimulation by TH (Yen, 2001)

2.22 Effects of thyroid hormones on the bones

Thyroid hormone is critical for normal bone growth and development. In children, hypothyroidism can cause short stature and delayed closure of the epiphyses. Biochemical studies have shown that TH can affect the expression of various bone markers in serum, reflecting changes in both bone formation and resorption (Yen, 2000). TH increases alkaline phosphatase and osteocalcin in osteoblasts. Additionally, osteoclast markers such as urinary hydroxyproline, urinary pyridinium, and deoxypyridinium cross-links are increased in hyperthyroid patients. These observations suggest that both osteoblast and osteoclast activities are stimulated by TH. Indeed, there is enhanced calcification and bone formation coupled to increased bone resorption in hyperthyroid patients (Yen, 2001). Additionally, the time interval between formation and subsequent mineralization of osteoid is shortened. The net effect on these bone cells is bone resorption and loss of trabecular bone thickness in hyperthyroidism. There also is marked increase in porosity and decreased cortical thickness in cortical bone in hyperthyroid patients (Baran and Braverman, 1991). These effects can lead to osteoporosis and

increased fractures.

TH may act on bone via TH stimulation of growth hormone and insulin-like growth factor I (IGF-I) or by direct effects on target genes (Yen, 2001).

2.23 Effects of thyroid hormones on body weight

Greatly increased thyroid hormone almost always decreases the body weight, and greatly decreased hormone almost always increases the body weight. These effects do not always occur, because thyroid hormone also increases the appetite, and this may counterbalance the change in the metabolic rate (Guyton and Hall, 2006).

2.24 Effects of thyroid hormones on metabolism

According to Cramer and McCall (1918), in rats, removal of the thyroid and the parathyroid glands does not produce any severe disturbance of the metabolism. There is at first a diminution of the total metabolism, which is followed later by a compensatory increase. The curves of the CO_2 excretion and O_2 intake and of the respiratory quotient do not differ in any essential points from those of a normal animal.

But according to Leonard (2003), Rhoades and Tanner (2004) and Guyton and Hall (2006), the thyroid gland exerts profound effects on oxidative metabolism in humans. The basal metabolic rate (BMR), which is a measure of oxygen consumption under defined resting conditions, is highly sensitive to thyroid status. A decrease in oxygen consumption results from a deficiency of thyroid hormones, and excessive thyroid hormone increases BMR. Oxygen consumption in all tissues except brain, testis, and spleen is sensitive to the thyroid status and increases in response to thyroid hormone.

2.25 Effects of thyroid hormones on lipid metabolism

Essentially all aspects of fat metabolism are also enhanced under the influence of thyroid hormone. In particular, lipids are mobilized rapidly from the fat tissue, which decreases the fat stores of the body to a greater extent than almost any other tissue element. This also increases the free fatty acid concentration in the plasma and greatly accelerates the oxidation of free fatty acids by the cells (Guyton and Hall, 2006)

Walters and McLean (1967) discovered that thyroidectomy lowered the incorporation of ^{14} C-labeled substrates into ^{14}C- labeled lipid, an effect further studied by the measurement of the activities of citrate -cleavage enzyme and acetate thiokinase. Restricting the food intake of the control rats to that of the thyroidectomised group lowered the activities of citrate-cleavage enzyme but no further depression was observed in thyroidectomy.

2.26 Thyroid function tests

The thyroid gland produces T_4 and T_3. But this production is not possible without stimulation from the pituitary gland (TSH) which in turn is also regulated by the hypothalamus's TSH Releasing Hormone. No one single laboratory test is 100% accurate in diagnosing all types of thyroid disease; however, a combination of two or more tests can usually detect even the slightest abnormality of thyroid function.
For example, a low T_4 level could mean a diseased thyroid gland or a non-functioning pituitary gland which is not stimulating the thyroid to produce T_4. Since the pituitary gland would normally release TSH if the T_4 is low, a high TSH level would confirm that the thyroid gland (not the pituitary gland) is responsible for the hypothyroidism (Norman, 2010).

If the T_4 level is low and TSH is not elevated, the pituitary gland is more likely to be the cause for the hypothyroidism. Of course, this would drastically effect the treatment since the pituitary gland also regulates the body's other glands (adrenals, ovaries, and testicles) as well as controlling growth in children and normal kidney function. Pituitary gland failure means that the other glands may also be failing and other treatment than just thyroid may be necessary. The most common cause for the pituitary gland failure is a tumour of the pituitary and this might also require surgery to remove.

The following tests reviewed by Norman (2010) are the commonly used tests for the function of the thyroid gland;

Measurement of Serum Thyroid Hormones

T_4 by RIA (radioimmunoassay) is the most used thyroid test of all. It is

frequently referred to as a T_7 which means that a resin T_3 uptake (RT_3u) has been done to correct for certain medications such as birth control pills, other hormones, seizure medication, cardiac drugs, or even aspirin that may alter the routine T_4 test. The T_4 reflects the amount of thyroxine in the blood. If the patient does not take any type of thyroid medication, this test is usually a good measure of thyroid function. The normal human adult range of T_4 in blood is 4 - 11 µg/Dl. Thyroxine (T_4) represents 80% of the thyroid hormone produced by the normal gland and generally represents the overall function of the gland. The other 20% is triiodothyronine measured as T_3 by RIA. Sometimes the diseased thyroid gland will start producing very high levels of T_3 but still produce normal levels of T_4. Therefore measurement of both hormones provides an even more accurate evaluation of thyroid function.

Thyroid Binding Globulin

Most of the thyroid hormones in the blood are attached to a protein called thyroid binding globulin (TBG). If there is an excess or deficiency of this protein it alters the T_4 or T_3measurement but does not affect the action of the hormone. If a patient appears to have normal thyroid function, but an unexplained high or low T_4, or T_3, it may be due to an increase or decrease of TBG. Direct measurement of TBG can be done and will explain the abnormal value. Excess TBG or low levels of TBG are found in some families as a hereditary trait. It causes no problem except falsely elevating or lowering the T_4 level. These people are frequently misdiagnosed as being hyperthyroid or hypothyroid, but they have no thyroid problem and need no treatment.

Measurement of Pituitary Production of TSH

Pituitary production of TSH is measured by a method referred to as IRMA (immunoradiometric assay). Normally, low levels (less than 5 units) of TSH are sufficient to keep the normal thyroid gland functioning properly. When the thyroid gland becomes inefficient such as in early hypothyroidism, the TSH becomes elevated even though the T_4 and T_3 may still be within the "normal"

range.This rise in TSH represents the pituitary glands response to a drop in circulating thyroid hormone; it is usually the first indication of thyroid gland failure. Since TSH is normally low when the thyroid gland is functioning properly, the failure of TSH to rise when circulating thyroid hormones are low is an indication of impaired pituitary function. The new "sensitive" TSH test will show very low levels of TSH when the thyroid is overactive (as a normal response of the pituitary to try to decrease thyroid stimulation). Interpretations of the TSH level depends upon the level of thyroid hormone; therefore, the TSH is usually used in combination with other thyroid tests such as the T_4 IA and T_3 IA.

TRH Test

In normal people TSH secretion from the pituitary can be increased by giving a shot containing TSH Releasing Hormone (TRH). A baseline TSH of 5 or less usually goes up to 10-20 after giving an injection of TRH. Patients with excess thyroid hormone (thyroxine or triiodothyronine) will not show a rise in TSH when given TRH. This "TRH test" is presently the most sensitive test in detecting early hyperthyroidism. Patients who show excessive response to TRH (TSH rises greater than 40) may be hypothyroid. This test is also used in cancer patients who are taking thyroid replacement to see if they are on sufficient medication. It is sometimes used to measure if the pituitary gland is functioning. The new "sensitive" TSH test has eliminated the necessity of performing a TRH test in most clinical situations.

Iodine Uptake Scan

A means of measuring thyroid function is to measure how much iodine is taken up by the thyroid gland (RAI uptake). Hypothyroid patients usually take up too little iodine and hyperthyroid patients take up excess iodine. The test is performed by giving a dose of radioactive iodine on an empty stomach. The iodine is concentrated in the thyroid gland or excreted in the urine over the next few hours. The amount of iodine that goes into the thyroid gland can be measured by a "Thyroid Uptake". Patients who are taking thyroid medication will not take up as much iodine in their thyroid gland because their own thyroid gland is not functioning. At other times the gland will concentrate iodine

normally but will be unable to convert the iodine into thyroid hormone; therefore, interpretation of the iodine uptake is usually done in conjunction with blood tests.

Thyroid Scan

Taking a scan of how well the thyroid gland is functioning requires giving a radioisotope to the patient and letting the thyroid gland concentrate the isotope (just like the iodine uptake scan above). Therefore, it is usually done at the same time that the iodine uptake test is performed. Although other isotopes, such as technetium, will be concentrated by the thyroid gland; these isotopes will not measure iodine uptake which is what we really want to know because the production of thyroid hormone is dependent upon absorbing iodine. It has also been found that thyroid nodules that concentrate iodine are rarely cancerous; this is not true if the scan is done with technetium. Therefore, all scans are now done with radioactive iodine. Pregnant women should not have thyroid scans performed because the iodine can cause development problems within the baby's thyroid gland.

Two types of thyroid scans are available. A camera scan is performed most commonly which uses a gamma camera operating in a fixed position viewing the entire thyroid gland at once. This type of scan takes only five to ten minutes. In the 1990's, a new scanner called a Computerized Rectilinear Thyroid (CRT) scanner was introduced. The CRT scanner utilizes computer technology to improve the clarity of thyroid scans and enhance thyroid nodules. It measures both thyroid function and thyroid size. A life-sized 1:1 color scan of the thyroid is obtained giving the size in square centimeters and the weight in grams. The precise size and activity of nodules in relation to the rest of the gland is also measured. CTS of the normal thyroid gland In addition to making thyroid diagnosis more accurate, the CRT scanner improves the results of thyroid biopsy. The accurate sizing of the thyroid gland aids in the follow-up of nodules to see if they are growing or getting smaller in size. Knowing the weight of the thyroid gland allows more accurate radioactive treatment in patients who have Graves' disease.

Thyroid Ultrasound

Thyroid ultrasound refers to the use of high frequency sound waves to obtain an image of the thyroid gland and identify nodules. It tells if a nodule is "solid" or a fluid-filled cyst, but it will not tell if a nodule is benign or malignant. Ultrasound allows accurate measurement of a nodule's size and can determine if a nodule is getting smaller or is growing larger during treatment. Ultrasound aids in performing thyroid needle biopsy by improving accuracy if the nodule cannot be felt easily on examination.

Thyroid Antibodies

The body normally produces antibodies to foreign substances such as bacteria; however, some people are found to have antibodies against their own thyroid tissue. A condition known as Hashimoto’s thyroidittis is associated with a high level of these thyroid antibodies in the blood. Whether the antibodies cause the disease or whether the disease causes the antibodies is not known; however, the finding of a high level of thyroid antibodies is strong evidence of this disease. Occasionally, low levels of thyroid antibodies are found with other types of thyroid disease. When Hashimoto's thyroiditis presents as a thyroid nodule rather than a diffuse goitre, the thyroid antibodies may not be present.

2.27 Pathophysiology of the thyroid

Thyroid disorders include hyperthyroidism (abnormally increased activity), hypothyroidism (abnormally decreased activity), genetic disorders and thyroid nodules, which are generally benign thyroid neoplasms, but may be thyroid cancers. All these disorders may give rise to goitre, that is, an enlarged thyroid.

Genetic Disorders Involving TH, TSH, or TRs

Resistance to TH is a genetic disorder caused by mutations in the TRb gene. Patients with this disorder have high TH levels and TSH levels, goitre (enlarged thyroid gland), and mild hypothyroid metabolisms. Clinical effects are less severe than with congenital hypothyroidism and can include short stature, delayed bone maturation, hyperactivity, learning disabilities, and hearing defects, as well as mixed features of hyper- and hypothyroidism. This condition is usually inherited dominantly.

Pendred's Syndrome is caused by a genetic defect that limits the incorporation of iodine into thyroid hormone, which wrecks the structure of the hormone. Pendred's Syndrome can cause hypothyroidism with goitre. The body compensates by producing more TSH and working harder to make enough thyroid hormone that works. The syndrome can also cause more serious problems, such as profound deafness, or non-syndromal deafness alone. These symptoms are present from birth. People who develop hypothyroidism later in life may have ringing in their ears and dulled hearing, but these symptoms are usually correctable by TH therapy, while deafness caused by Pendred's Syndrome is not.

TSH receptor (thyrotropin receptor) gene mutations often cause hyperthyroidism, or TSH insensitivity, which leads to normal TH levels in the blood with elevated TSH levels. TSH has unknown effects on lymphocytes and brain cells; therefore imbalances affecting TSH levels may cause additional, unknown effects on the brain and immune system. One mutation was found in association with Graves' disease. Graves' disease is an autoimmune form of hyperthyroidism, and the genes that seem to increase risk of Graves' disease are associated with immunity.

In humans, thyroid hormone plays a notable role in brain development from the middle of pregnancy to the second year of life. Maternal or foetal hypothyroidism, whether caused by lack of iodine during the pregnancy, or by other problems, can cause a non-genetic condition called cretinism. Babies affected by cretinism can develop normal intelligence if the condition is remedied within a few months, but otherwise they suffer severe, irreversible mental retardation. One severe type of cretinism can also be caused by mutations in the TRα gene, and may be untreatable.

Hyperthyroidism

Hyperthyroidism, or overactive thyroid, is the overproduction of the thyroid hormones T_3 and T_4, and is most commonly caused by the development of Graves' disease, an autoimmune disease in which antibodies are produced which stimulate the thyroid to secrete excessive quantities of thyroid hormones. The disease can result in the formation of a toxic goitre as a result

of thyroid growth in response to a lack of negative feedback mechanisms. Hyperthyroidism causes accelerated heart rate and fatigue, even when patients are at rest. It produces lower exercise tolerance because protein and fat catabolism are accelerated, resulting in build-up of ketones. Hyperthyroid people often show a fine tremor in their hands. They have higher resting heart rates, but not higher maximum heart rates for exercise, in comparison to normal subjects. Some experience thyroid storms--high overloads of thyroid hormones that accelerate their heart rate to as high as 300 beats a minute. This is a very life-endangering condition and can result in arrhythmia or heart attack (Phillips, 2012).

Beta blockers are used to decrease symptoms of hyperthyroidism such as increased heart rate, tremors, anxiety and heart palpitations, and anti-thyroid drugs are used to decrease the production of thyroid hormones, in particular, in the case of Graves' disease. These medications take several months to take full effect and have side effects such as skin rash or a drop in white blood cell count, which decreases the ability of the body to fight off infections. Alternatively, the gland may be partially or entirely removed surgically, though iodine treatment is usually preferred since the surgery is invasive and carries a risk of damage to the parathyroid glands or the nerves controlling the vocal cords. If the entire thyroid gland is removed, hypothyroidism results.

Hypothyroidism

Hypothyroidism is the underproduction of the thyroid hormones T_3 and T_4. Hypothyroid disorders may occur as a result of congenital thyroid abnormalities, autoimmune disorders such as Hashimoto's thyroiditis or iodine deficiency (more likely in poorer countries) or the removal of the thyroid following surgery to treat severe hyperthyroidism and/or thyroid cancer. Some of the most profound effects of thyroid hormone imbalance are in the mental arena. Hypothyroid people sleep easily and do not get full refreshment from their sleep. During waking hours, they experience fatigue, apathy, and "brain fog" (short-term memory problems and attention deficits). These problems may affect their daily functioning and cause increased stress and depression.

Thyroid hormone acts as a neurotransmitter. Thyroid hormone imbalance can

mimic psychiatric disease because T_3 influences levels of serotonin, a neurotransmitter integral to moods and behaviour. Low levels of T_3 can cause depression. Some anti-depressants make hypothyroid patients feel even worse because the medications depress T_3 levels. Paradoxically, some substances labelled depressants such as alcohol or opiates can increase T_3 levels by impairing the breakdown of T_3 in the brain, thus lifting mood. This may be one reason why these substances are so addictive.

Severe hypothyroidism can cause symptoms similar to Alzheimer's disease: memory loss, confusion, slowness, paranoid depression, and in extreme stages, hallucinations. Thyroid disease is one of the many treatable diseases that must be ruled out before arriving at the diagnosis of Alzheimer's, which is incurable and cannot be definitely diagnosed until after death. Risk of hypothyroidism increases with age; by age 60, 17% of women and 9% of men have symptoms of thyroid disease.

Low thyroid hormone levels also produce fatigue, slight hypoglycemia (low blood sugar), slowed digestion of food, and constipation. Infertility is common. These symptoms can indicate that other diseases are present, particularly because TH levels tend to go down during prolonged illness in an effort to conserve energy. Chronic disease, such as Lyme disease, can mimic (or cause) hypothyroidism.

Hypothyroidism is treated with hormone replacement therapy, such as levothyroxine, which is typically required for the rest of the patient's life. Thyroid hormone treatment is given under the care of a physician and may take a few weeks to become effective.

Negative feedback mechanisms result in growth of the thyroid gland when thyroid hormones are being produced in sufficiently low quantities as a means of increasing the thyroid output; however, where the hypothyroidism is caused by iodine insufficiency, the thyroid is unable to produce T_3 and T_4 and as a result, the thyroid may continue to grow to form a non-toxic goitre. It is termed non-toxic as it does not produce toxic quantities of thyroid hormones, despite its size.

2.28 Causes of thyroid disease

Thyroid diseases are among the most prevalent of medical conditions and their manifestations vary considerably and are determined principally by the availability of iodine (Vanderpump, 2018). The most common cause of thyroid disorders worldwide is iodine deficiency due to absence of adequate iodine in the diet or high intake of goitrogenic foods leading to goitre formation and hypothyroidism (Vanderpump, 2018).

In iodine sufficient regions, the autoimmune thyroid diseases; Hashimoto's thyroiditis and Grave's disease are the most clinical entities affecting the thyroid. The most common cause of hypothyroidism is Hashimoto's thyroiditis and the most frequent cause of hyperthyroidism is Grave's disease (Kopp, 2002). Some experts believe that continual iodine overdoses leads to autoimmune thyroid disease, because it seems to be the major cause of thyroid disorder in developed countries.

Autoimmune thyroid disease is identified by detecting antibodies in the blood. In the case of Graves' disease, antibodies latch onto an enzyme essential for making T_4, and keep it active and continually turned on. Graves' disease is treated by suppressing or killing (removing) the thyroid and then stabilizing the patient on thyroid hormone replacements. In Hashimoto's thyroiditis, antibodies latch onto the same enzyme, but block its function, and help trigger destruction of the thyroid. In the early stages of Hashimoto's thyroiditis, the thyroid may produce too much TH, but as the thyroid is slowly destroyed, the patients TH levels drop. Hashimoto's thyroiditis is treated with thyroid hormone replacements.

Pollutant chemicals like polychlorinated biphenyls (PCBs) and dioxins have been shown to interfere with thyroid function and are more prevalent in industrialized countries where thyroid disease levels are high. Autoimmune thyroid disease, either hyperthyroidism or hypothyroidism, is also linked to post-traumatic stress disorder and is often first observed clinically after periods of prolonged stress.

2.29 Goitrogenic foods

Goitrogenic foods are foods that contain goitrogens which suppress the function of the thyroid gland by interfering with iodine uptake, and can as a result, cause hypothyroidism and goitre.

They include; soybeans and soy products, cassava and its products, peanuts and cruciferous vegetables such as cabbage, broccoli, cauliflower, brussel sprouts, mustard, rutabagas, kohlrabi and turnips.

2.30 *Glycine max* (soybeans)

Soybean (Glycine max) is a legume species native to East Asia, widely grown for its edible bean which has numerous uses. It is now grown commercially in many parts of the world, and soybeans are a globally important crop, providing oil and protein (Anders, 2013). Soybean is among the major industrial and food crops grown in every continent. It has been successfully grown in many states in Nigeria using low agricultural input.

2.31 Constituents of soybeans

Together, soybean oil and protein content account for about 60% of dry soybeans by weight (protein at 40% and oil at 20%). The remainder consists of 35% carbohydrate and about 5% ash. Soybean consists of approximately 8% seed coat or hull, 90% cotyledons and 2% hypocotyl axis or germ.

Anti-nutritional elements

Soybeans contain a number of elements which have unwanted or detrimental effects in the human organism. Anti-nutrients found in soybeans include;

Phytic acid or the salt form phytate acts as an antioxidant, and furthermore has a strong binding affinity to important minerals, such as calcium, iron, and zinc, although the binding of calcium with phytic acid is pH-dependent and ascorbic acid (vitamin C) can reduce phytic acid's effect on iron (Isanga and Zhang, 2008).

Trypsin inhibitors are molecules, which bind to the enzyme trypsin, and thereby inhibit the degradation of certain lipid bonds among amino acids (lysine and arginine) of proteins in the intestine of humans and animals. This prevents the

uptake of amino acids, and further detrimentally affects the enzyme balance and may cause hypertrophic pancreas responses. Soy beans contain at least two types of trypsin inhibitors, and the biological function of the inhibitors is thought to be, that animals will avoid eating the raw beans in the long run (Selgrade et al., 2009). Heating, fermentation and leaching eliminates the activity of the inhibitors (Isanga and Zhang, 2008).

Lectins are a natural part of many legumes, and are toxic to humans at higher levels. In mature raw soybeans the lectin levels are low, whereas in green immature beans the levels may be higher. Lectins bind to the gut wall and reduce epithelial cell vitality and functionality, and thus inhibit the absorption of vital nutritional elements into the body. Possible beneficial effects of lectins, e.g. their anti-cancer effects, have, however, been debated in science (Isanga and Zhang, 2008). Leaching, cooking and (moist) heat treatment deactivates the lectins (Isanga and Zhang, 2008). - Soluble carbohydrates are broken down during fermentation, soy concentrate, soy protein isolates, tofu, soy sauce, and sprouted soybeans are without flatus activity. On the other hand, there may be some beneficial effects to ingesting oligosaccharides such as raffinose and stachyose, namely, encouraging indigenous bifidobacteria in the colon against putrefactive bacteria

Isoflavones

Isoflavones are a group of naturally occurring heterocyclic phenols, which are present in soy bean at levels of 0.1 to 5 mg/g (Isanga and Zhang, 2008). The three major groups of isoflavones found in soybeans are genistein, daidzein, and glycitein, and the genistein and daidzein forms constitute the absolutely largest proportion of isoflavones in soy.

In in-vitro studies and in rats, isoflavones have been shown to compete for an enzyme that is used to make thyroid hormone and partially inactivate thyroid peroxidase, an enzyme required for the synthesis of thyroid hormones. However, not only is the rat extremely sensitive to goitrogenic problems in comparison to humans, but despite inhibiting enzyme activity, soy-containing diets allow normal thyroid function. Soy may somewhat inhibit the absorption of synthetic thyroid hormone, such as synthroid, which is taken by hypothyroid

patients. However, foods in general have this effect, as do fibre-rich foods, herbs and many drugs. For this reason, thyroid hormone is taken on an empty stomach and hypothyroid patients can still consume soy products. If there is any small effect on absorption, the medication dose can easily be adjusted accordingly.

2.32 Uses of soybeans

Soybeans are very nutritious and contain an average of 40% protein. It can be used directly for food in the household or processed for soymilk, dadawa, soy-cheese,, cooking oil and a range of other products including tom brown (infant complementary food), soybean cake (for poultry feed), the haulms provide good feed for sheep and goats.

2.33 *Manihot esculenta crantz* (Cassava)

Cassava (*Manihot esculenta Crantz*) is a starch root tuber. It is a basic staple food vital for the livelihood of up to 500 million farmers and countless processors and traders around the world (Plucknett *et al*., 2000). The plant is very robust, resistant to drought and cassava production does not require high inputs. Cassava originated in South America where it was domesticated 2,000-4,000 years B.C. and was introduced into Africa in the 16th century by the Portuguese (Jones, 1959; Fauquet and Fargette, 1990). In the 18th century it was introduced to the east coast of Africa. The total world cassava production in the year 2002 was 184,852,540 metric tonnes, with 100,689,149 being produced in Africa alone (FAO, 2003).

2.34 Constituents of cassava

Processing cassava by peeling, chopping, and cooking reduces its nutritional value because many of the vitamins are destroyed by processing as well as most of the fibre and resistant starch (Elliot, 2017). Therefore, the more popular processed forms of cassava such as tapioca and garri have very limited nutritional value (Elliot, 2017).

A 100gram serving of boiled cassava root contains 112 calories (98% from carbs and the rest from a small amount of protein and fat). The following nutrients are found in 100grams of boiled cassava; calories (112), Carbohydrate (27grams), fibre (1gram), thiamine (20% of RDI), Phosporus (5%

of RDI), Calcium (2% of RDI) and ribloflavin (2% of the RDI) . It also contains small amounts of iron, vitamin C and niacin.

Resistant starch

Cassava is high in resistant starch which is a type of starch which bypasses digestion and has properties similar to soluble fibre. The benefits of resistant starch are promising, but it is important to note that many processing methods may lower cassava's resistant starch content (Elliot, 2017).

Anti-nutrients

Anti-nutrients are plant compounds that interfere with digestion and inhibit absorption of vitamins and minerals in the body. They are more likely to impact populations at risk of malnutrition which include populations that rely on cassava as the major source of calories. Anti-nutrients found in cassava include; saponins, phytate and tannins

Cyanide

Manihot esculenta crantz (Cassava tuber) products contain cyanide (McPhee *et al*., 2009) which is digested to form thiocyanate. This compound can block thyroidal uptake of iodine by competing with sodium iodide symporter (NIS). A report by Chandra *et al*., (2006) on an experiment which lasted for 90 days stated that 'After consumption, cyanogenic constituents in cassava are metabolized to thiocyanate and iodine-retaining capacity seems to be dependent on thiocyanate exposure. Frequent consumption can increase the risk of cyanide poisoning which impair thyroid function and nerve function and its associated paralysis and organ damage. Since protein helps to get rid of cyanide, cyanide poisoning is of greater concern for those who suffer from protein deficiencies and depend on cassava as a major source of calories.

Cassava is generally safer for consumption when it is eaten occasionally in moderate amounts (1/3 to ½ cup) (Elliot, 2017). Proper processing such as peeling, soaking for 48-60 hours, cooking (by boiling, roasting or baking) and paring with protein in meals (Elliot, 2017).

2.35 Uses of cassava

Cassava is the chief source of dietary food energy for the majority of the people living in the lowland tropics and much of the sub-humid tropics of west and central Africa. Cassava products are important for livestock feed formulation, cassava starch, cassava flour, cassava juice and fermented cassava are used in industries. Cassava starch is used for making pastries derivatives, jellies, thickening agents, gravies, custard powder, baby food, glucose and confectioneries. Cassava starch is used in making paste products such as spaghetti and macaroni as well as brewery products such as beer and ethanol in combination with sugarcane. Fermented cassava is used in preparation of garri, fufu, tapioca, (Echebiri and Edaba, 2008).

2.36 Glucose

Glucose is a monosaccharide with formula $C_6H_{12}O_6$ or H-(C=O)-$(CHOH)_5$-H, whose five hydroxyl (OH) groups are arranged in a specific way along its six-carbon backbone. Cells use it as the primary source of energy and a metabolic intermediate (Clark and Sorkoloff, 1999).

Glucose exists in several different structures, but all of these structures can be divided into two families of mirror-images (stereoisomers). Only one set of these isomers exists in nature, those derived from the "right-handed form" of glucose, denoted D-glucose. D-glucose is often referred to as dextrose. According to the Merriam-Webster online dictionary, the term dextrose is derived from dextrorotatory glucose. Solutions of dextrose rotate polarized light to the right. Starch and cellulose are polymers derived from the dehydration of D-glucose. The other stereoisomer, called L-glucose, is hardly found in nature.

In its fleeting open-chain form, the glucose molecule has an open (as opposed to cyclic) and unbranched backbone of six carbon atoms, C-1 through C-6; where C-1 is part of an aldehyde H(C=O)-, and each of the other five carbons bears one hydroxyl group -OH. The remaining of the backbone carbons are satisfied by hydrogen atoms -H. Therefore glucose is an hexose and an aldose, or an aldohexose.

Each of the four carbons C-2 through C-5 is chiral, meaning that its four bonds

connect to four different substituents. (Carbon C-2, for example, connects to -(C=O)H, -OH, -H, and -(CHOH)4H.) In D-glucose, these four parts must be in a specific three-dimensional arrangement. Namely, when the molecule is drawn in the Fischer projection, the hydroxyls on C-2, C-4, and C-5 must be on the right side, while that on C-3 must be on the left side.

The positions of those four hydroxyls are exactly reversed in the Fischer diagram of L-Glucose. D- and L-glucose are two of the 16 possible aldohexoses; the other 14 are allose, altrose, mannose, gulose, idose, galactose, and talose, each with two isomers, 'D-' and 'L-'.

2.37 Physical properties of glucose

Solutions

All forms of glucose are colourless and easily soluble in water, acetic acid, and several other solvents. They are only sparingly soluble in methanol and ethanol.

The open-chain form is thermodynamically unstable, and it spontaneously tautomerizes to the cyclic forms. (Although the ring closure reaction could in theory create four- or three-atom rings, these would be highly strained and are not observed.) In solutions atroom temperature, the four cyclic isomers interconvert over a timescale of hours, in a process called muta-rotation (John, 1988) Starting from any proportions, the mixture converges stable ratio of α:β 36:64. The ratio would be α:β 11:89 if it were not for the influence of the anomeric effect (Juarista *et al.*, 1995). Muta-rotation is considerably slower at temperatures close to 0°C.

Muta-rotation consists of a temporary reversal of the ring-forming reaction, resulting in the open-chain form, followed by a re-forming of the ring. The ring closure step may use a different -OH group than the one recreated by the opening step (thus switching between pyranose and furanose forms), and/or the new hemiacetal group created on C-1 may have the same or opposite handedness as the original one (thus switching between the α and β forms). Thus, even though the open-chain form is barely detectable in solution, it is an essential component of the equilibrium.

Solid state

Depending on conditions, three major solid forms of glucose can be crystallised from water solutions: α-glucopyranose, β-glucopyranose, and β-glucopyranose hydrate (Fred *et al.*, 2006).

2.38 Glucose Homeostasis

Gluco-regulation is the maintenance of steady levels of glucose in the body; it is part of homeostasis, and so keeps a constant internal environment around cells in the body.

The hormone insulin is the primary regulatory signal in animals, suggesting that the basic mechanism is very old and very central to animal life. When present, it causes many tissue cells to take up glucose from the circulation, causes some cells to store glucose internally in the form of glycogen, causes some cells to take in and hold lipids, and in many cases controls cellular electrolyte balances and amino acid uptake as well. Its absence turns off glucose uptake into cells, reverses electrolyte adjustments, begins glycogen breakdown and glucose release into the circulation by some cells, begins lipid release from lipid storage cells, etc. The level of circulatory glucose (known informally as "blood sugar") is the most important signal to the insulin-producing cells. Diet controls major aspects of metabolism via insulin, because the level of circulatory glucose is largely determined by the intake of dietary carbohydrates. In humans, insulin is made by beta cells in the pancreas, fat is stored in adipose tissue cells, and glycogen is both stored and released as needed by liver cells. Regardless of insulin levels, no glucose is released to the blood from internal glycogen stores from muscle cells.

The hormone glucagon, on the other hand, has an effect opposite to that of insulin, forcing the conversion of glycogen in liver cells to glucose, which is then released into the blood. Muscle cells, however, lack the ability to export glucose into the blood (Leonard *et al.*, 2005). The release of glucagon is precipitated by low levels of blood glucose. Other hormones, notably growth hormone, cortisol, and certain catecholamines (such as epinepherine) have glucoregulatory actions similar to glucagon. Amino acids also stimulate secretion (though they also increase glucagon release).

Hormones: Gastrointestinal hormones stimulate secretion (gastrin, secretin,

CCK). Several hormones secreted by the gastrointestinal tract, including gastric inhibitory peptide (GIP), gastrin, and secretin, promote insulin secretion. An oral dose of glucose produces a greater increment in insulin secretion than an equivalent intravenous dose because oral glucose promotes the secretion of GI hormones that augment insulin secretion by the pancreas. Direct infusion of acetylcholine into the pancreatic circulation stimulates insulin secretion, reflecting the role of parasympathetic innervations in regulating insulin secretion. Adrenaline, noradrenaline and somatostatin inhibit insulin release. In addition to factors that stimulate insulin secretion, there are several potent inhibitors. Exogenously administered somatostatin is a strong inhibitor. It is presumed that pancreatic somatostatin plays a role in regulating insulin secretion, but the importance of this effect has not been fully established. Epinephrine and norepinephrine, the primary catecholamines, are also potent inhibitors of insulin secretion. This response would appear appropriate because during periods of stress and high catecholamine secretion, the desired response is mobilization of glucose and other nutrient stores. Insulin generally promotes the opposite response, and by inhibiting insulin secretion, the catecholamines produce their full effect without the opposing actions of insulin (Rhoades and Tanner, 2003).

Hormones that stimulate glucagon synthesis and secretion are the catecholamines; adrenaline and noradrenaline. Glucagon synthesis and secretion is inhibited by somatostatin and insulin.

2.39 Glucose Tolerance Test

According to the Oxford Dictionary of Biochemistry, glucose tolerance test is any procedure designed to assess the response of an individual to a loading dose of glucose, widely used in the diagnosis of diabetes mellitus. The standard oral glucose tolerance test is performed in the morning after 12 hours of fasting following at least 3 days' unrestricted diet, physical activity, and freedom from medication. After collection of the fasting blood sample, 75 g (or 1.75g per kg of body weight for children) glucose is ingested in 5 minutes in 200 — 500ml water. Blood samples are then collected at 30-minute intervals for 2 hours, and urine samples are collected at 0, 1, and 2 hours. All samples are assayed for glucose. For capillary blood samples, a maximum glucose

level below 10mmol/l (180mg/100 ml) and a 2-hour value less than 7.5mmol/l (135mg/100ml) are the criteria for normality. If venous blood is used the corresponding values are 8.9mmol/l (160mg/100ml) and 6.1mmol/l (110mg/100ml). The urine samples should be glucose free.

Table 2.1. Table showing 1999 WHO Diabetes criteria - Interpretation of Oral Glucose Tolerance Test

Glucose Levels	Normal	Impaired Fasting Glycaemia (IFG)	Impaired glucose tolerance(IGT)	Diabetes Mellitus (DM)

Venous Plasma	Fasting	2hours	Fasting	2hours	fasting	2hours	Fasting	2hours
(mmol/l)	<6.1	<7.8	≥6.I&<7.0	<7.8	<7.0	≥7.8	≥7.0	≥11.1
(mg/dl)	<110	<140	≥110&<126	<140	<126	≥140	≥126	≥200

From table 2.1 in the previous page, it can be seen that oral glucose tolerance test can be used to determine the following conditions;

Diabetes mellitus

Impaired glucose tolerance

Impaired fasting glycaemia

2.40 Homeostatic Model Assessment of Insulin Resistance (HOMA-IR)

This is a method that is used in quantifying insulin resistance. It was first described by using the name; HOMA by Mathew *et al.*, in 1985. Originally, it was designed as a special case of a more general structure (HOMA_CIGMA) that includes the continuous infusion of glucose with model assessment (CIGMA) approach. Both techniques use mathematical equations to describe the functioning of the major effector organs influencing glucose and insulin interactions. This model has been tested severally against other measures of insulin resistance. (Mathew *et al.*, 1985)

2.41 Insulin Tolerance Test

According to the method of (Jing Ai *et al.*, 2005),

1. Rats are fasted for six hours.
2. Each rat is weighed and insulin dose is calculated 0.05µ/kg body weight. Insulin is prepared at 0.1µ/ml in advance (16.6µl of 10mg/ml insulin in 40ml PBS).
3. Blood glucose is measured for each rat to get the value for 0 time point. Blood glucose level will be determined by glucometre in blood from tail vein. Tail snipping is used to get blood.
4. Insulin is injected intra- peritoneally
5. Blood glucose is measured at 30, 60, 90 and 120 minutes after the insulin injection.

2.42 The pancreas

The pancreas is located in the upper left quadrant of the abdominal cavity extending from the curve of the duodenum to the spleen (Leonard, 2005). The pancreas is composed of two major types of tissues;

(1) The acini, which secrete digestive juices into the duodenum, and

(2) The islets of Langerhans, which secrete insulin and glucagon directly into the blood.

One to two million highly vascularised and richly innervated islets comprise the

endocrine pancreas. These islets contain insulin-secreting beta cells, glucagon -secreting alpha cells, and somatostatin-secreting delta cells. The beta cells make up about 60% of all islet cells and lie mainly in the middle of the islet cells. The beta cells secrete insulin and amylin.

The apha cells which make up about 25% of the islet cells secrete glucagon.

The delta cells which comprise about 10% of the islet cells secret somatostatin.

2.43 Glucagon

This is a large polypeptide composed of a chain of 29 amino acids. It is secreted by the alpha cells of the Islets of Langerhans. Its overall effect is to increase blood glucose concentration when the blood glucose concentration is low.

Alpha cells respond directly to blood glucose concentrations. Glucagon secretion is maximal at low blood glucose concentrations and inhibited at high glucose concentrations;

Increased concentrations of amino acids in blood and the gut hormone cholecystokinin stimulate glucagon secretion;

Its physiologic role is to stimulate hepatic production of metabolic fuels and increase blood concentrations of glucose and ketone bodies by;

- **Breakdown of stored glycogen (glycogenolysis):** It does this by a cascade of events. Glucagon activated adenyl cyclase in the hepatic cell membrane which causes the formation of cyclic AMP which activates protein kinase which activates phosphorylase b kinase which converts phosphorylase b into phosphorylase a which promotes the degredation of glycogen to glucose-1-phosphate which is then phosphorylated and the glucose is released from the liver cells.

- **New synthesis of glucose (gluconeogenesis) in the liver**: glucagon increases amino acid uptake by liver cells which is then converted to

glucose by gluconeogenesis. This is achieved by activating the enzyme system for converting pyruvate to phophoenolpyruvate. Other effects are; it activates adipose cell lipase making fatty acids available for the energy systems of the body. It inhibits storage of triglycerides in the liver

- **It also inhibits glycogenesis**

Somatostatin, insulin, ketone bodies, and free fatty acids inhibit glucagon secretion.

2.44 Insulin

Insulin was first isolated from the pancreas by Banting and Best in 1922. The discovery of insulin changed the fate of diabetic patients from being severe to almost normal.

Insulin is a small protein; human insulin has a molecular weight of 5808. It is composed of two amino acid chains, connected to each other by disulfide linkages. Insulin is synthesised in the beta cells of the islets of langerhans. Synthesis of insulin starts with the translation of insulin RNA by ribosomes to form preproinsulin which has a molecular weight of 11,500 and is cleaved in the endoplasmic reticulum to generate proinsulin which has a molecular weight of about 9000 and is made up of three peptide chains A, B and C. In the golgi apparatus, proinsulin is further cleaved to form insulin which is made up of A and B chains connected by C peptide. C peptide and insulin are stored in the secretory granules and are secreted in equimolar quantities. Between 5-10 percent of the final product secreted is proinsulin.

Insulin that is secreted into the bloodstream exists almost completely in unbound form with a half life of about 6minutes. Save the portion of insulin that combines with receptors in the target cells, the remainder is degraded by the enzyme insulinase mainly in the liver and also in the kidney and muscles. This rapid degradation and removal of insulin is very important because at times, it is necessary to rapidly turn off the control functions of insulin.

In order for insulin to carry out its effects on target cells it must first bind with and activate a membrane receptor .The insulin membrane receptor protein has a molecular weight of 300,000. When the receptor is activated, it causes the

subsequent effects.

The insulin receptor comprises four subunits held together by disulfide linkages; two alpha subunits and two beta subunits. The alpha subunits lie entirely outside the cell membrane while the beta subunits penetrate the cell membrane all the way into the cytoplasm.

Insulin binds with the alpha subunits outside the cell membrane which leads to auto-phosphorylation of the beta subunits as a result of their linkage with the alpha subunits to which insulin is bound. Auto-phosphorylation of the beta subunits causes activation of a local tyrosine kinase which then leads to phosphorylation of multiple other intracellular enzymes e.g insulin-receptor substrates (IRS) which are expressed in different tissues.

Insulin directs intracellular metabolic machinery to produce desired effects on carbohydrate fat and protein metabolism. The end effects of insulin stimulation are the following

After insulin binds with the receptors, about 80 percent of the cells especially the muscle and adipose tissue cells in the body increase their uptake of glucose from the blood. This glucose is rapidly phosphorylated to become a substrate for carbohydrate metabolism.

Insulin secretion is activated by increased blood glucose as occurs immediately after a meal.

The principal physiologic action of insulin is to promote storage of metabolic fuels and reduce blood glucose level by:

- increasing uptake by increasing permeability of the cell membrane to glucose to glucose and use of glucose by muscle and connective tissue cells
- Insulin increases glucose transport and It activates conversion of glucose to glycogen (glycogenesis) in the muscles and liver
- Inhibition of glucose production (gluconeogenesis) from protein and fat.
- Inhibition of the breakdown of glycogen (glycogenolysis)

- It also promotes the synthesis of fatty acids and storage of fat in adipose tissues (lipogenesis) and promotes the uptake of amino acids by cells and the synthesis of protein.

2.45 Insulin Resistance

Insulin resistance is reduced sensitivity of tissues to insulin mediated biological acitivity (Alan, 2012). Development of insulin resistance and impaired glucose metabolism is usually a gradual process, beginning with excess weight gain and obesity (Hall, 2008). The mechanisms that link obesity with insulin resistance, however, are still uncertain. Some studies suggest that there are fewer insulin receptors, especially in the skeletal muscle, liver, and adipose tissue, in obese than in lean subjects (Hall, 2008). However, most of the insulin resistance appears to be caused by abnormalities of the signaling pathways that link receptor activation with multiple cellular effects. Impaired insulin signaling appears to be closely related to toxic effects of lipid accumulation in tissues such as skeletal muscle and liver secondary to excess weight gain (Hall, 2008)

Insulin resistance is part of a cascade of disorders that is often called the "metabolic syndrome." Some of the features of the metabolic syndrome include: obesity, especially accumulation of abdominal fat, insulin resistance, fasting hyperglycemia, lipid abnormalities such as increased blood triglycerides and decreased blood high-density lipoprotein-cholesterol and hypertension. All of the features of the metabolic syndrome are closely related to excess weight gain, especially when it is associated with accumulation of adipose tissue in the abdominal cavity around the visceral organs. The role of insulin resistance in contributing to some of the components of the metabolic syndrome is unclear, although it is clear that insulin resistance is the primary cause of increased blood glucose concentration (Hall, 2008)

2.46 Somatostatin

The physiologic importance of pancreatic somatostatin is not understood. Because it can inhibit secretion of both insulin and glucagon, it has been suggested that somatostatin, by acting in a paracrine fashion, may contribute to the regulation of glucagon and insulin secretion.

Increased concentrations of glucose or amino acids in blood stimulate somatostatin secretion by intestinal delta cells.

2.47 Diabetes Mellitus

Diabetes mellitus is a disease of metabolic dysregulation most notably a dysregulation of glucose metabolism accompanied by long-term vascular and neurological complications (Rhoades and Tanner, 2003). Diabetes mellitus, often simply referred to as diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), unintended weight loss, polydipsia (increased thirst) and polyphagia (increased hunger) (Wild *et al.*, 2004).

The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. The prevalence of diabetes is higher in men than women, but there are more women with diabetes than men. The urban population in developing countries is projected to double between 2000 and 2030. The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people above 65 years of age. (Wild *et al.*, 2004)

There are two main types of diabetes:

Type 1 diabetes:

Results from the body's failure to produce insulin, and presently requires the person to inject insulin. (also referred to as insulin-dependent diabetes mellitus(IDDM) and juvenile diabetes.)

Type 1 diabetes mellitus is characterized by absolute insulin deficiency. In type 1A, a cellular-mediated autoimmune destruction of beta cells of the pancreas occurs. The disease process is initiated by an environmental factor that is, an infectious or non-infectious agent in genetically susceptible individuals (Olatunbosun 2011).

Some genes in the histocompatibility leukocyte antigen (HLA) system are thought to be crucial. A stress-induced epinephrine release, which inhibits insulin release (with resultant hyperglycemia), sometimes occurs and may be followed by a transient asymptomatic period known as "the honeymoon." Lasting weeks to months, the honeymoon precedes the onset of overt, permanent diabetes (Olatunbosun and Dagogo-Jack, 2011).

Amylin, a beta-cell hormone that is normally co-secreted with insulin in response to meals, is also completely deficient in persons with type 1 diabetes mellitus. Amylin exhibits several gluco-regulatory effects that complement those of insulin in postprandial glucose regulation. Idiopathic forms of type 1 diabetes also occur, without evidence of autoimmunity or HLA association; this subset is termed type 1B diabetes.

In a state of health, normoglycemia is maintained by fine hormonal regulation of peripheral glucose uptake and hepatic production.

Type 2 diabetes

This type of diabetes mellitus results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency. It was formerly referred to as non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes. Type 2 diabetes mellitus results from a defect in insulin secretion and an impairment of insulin action in hepatic and peripheral tissues, especially muscle tissue and adipocytes (Corpeleijn *et al.*, 2008). A post-receptor defect is also present, causing resistance to the stimulatory effect of insulin on glucose use. As a result, a relative insulin deficiency develops, unlike the absolute deficiency found in patients with type 1 diabetes. The specific etiologic factors are not known, but genetic input is much stronger in type 2 diabetes than in the type 1 form (Defronzo, 2004)

Impaired glucose tolerance is a transitional state from normoglycemia to frank diabetes, but patients with impaired glucose tolerance exhibit considerable heterogeneity. Type 2 diabetes, or glucose intolerance, is part of a dysmetabolic syndrome (syndrome X) that includes insulin resistance,

hyperinsulinemia, obesity, hypertension, and dyslipidemia. Current knowledge suggests that the development of glucose intolerance or diabetes is initiated by insulin resistance and worsened by the compensatory hyperinsulinemia (Olatunbosun and Dagogo-Jack 2011).

The progression to type 2 diabetes is influenced by genetics and environmental or acquired factors, such as a sedentary lifestyle and dietary habits that promote obesity. Most patients with type 2 diabetes are obese, and obesity is associated with insulin resistance. Central adiposity is more important than increased generalized fat distribution. In patients with frank diabetes, glucose toxicity and lipotoxicity may further impair insulin secretion by the beta cells (Dagogo-Jack, 1997) (Li*et al.*, 2010) (Ko *et al.*, 2010).

2.48 Effects of Thyroid Hormones on Glucose Homeostasis

T_3 accelerates virtually all aspects of metabolism including carbohydrate utilization. It increases glucose absorption from the digestive tract, glycogenolysis and gluconeogenesis in hepatocytes, and glucose oxidation in liver, fat, and muscle cells (Leonard, 2005). No single or unique reaction in any pathway of carbohydrate metabolism has been identified as the rate-determining target of T_3 action. Rather, carbohydrate degradation appears to be driven by other factors, such as increased demand for ATP, the content of carbohydrate in the diet, or the nutritional state. Although T_3 may induce synthesis of specific enzymes of carbohydrate and lipid metabolism, e.g., the malic enzyme, glucose-6-phosphate dehydrogenase, and 6-phosphogluconatedehydrogenase, it appears to behave principally as an amplifier working in conjunction with other signals.

The study of the respiratory quotient and of the urine by Cramer and McCall in 1918 showed that removal of the thyroid gland does not impair the power of the cells of the organism to oxidise carbohydrates. From this it can be inferred that the condition of the carbohydrate metabolism in experimental hyperthyroidism is not due to a direct stimulating effect of the thyroid hormone on the oxidation of carbohydrates.

A study by Walters and McLean (1967) revealed that thyroidectomy depressed the oxidation of ^{14}-C labeled substrates, however, the ratio of oxidation of I-^{14}C

glucose/ oxidation of 6-^{14}C glucose was unaltered. They also discovered that the activities of hexokinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and NADP-linked isocitrate dehydrogenase were all lower in the thyroidectomised group than in the pair-fed control group.

In hyperthyroid patients, thyroid hormone levels are inversely related to the rate of insulin release, suggesting a relationship between altered insulin secretion and severity of hyperthyroidism (Roubsanthisuk *et al.*, 2006).

The effects of excess thyroid hormone on various organ systems results in altered glucose metabolism, potentially leading to diabetic ketoacidosis and thyroid storm. Thyroid hormone increases hepatic glucose output, decreases peripheral glucose disposal, increases inactive insulin secretion by the pancreas, and increases insulin clearance by the kidney.

In addition to these secretion and clearance alterations, overweight hyperthyroid women lose their first-phase response to hyperglycemia, demonstrating lower insulin peaks after intravenous glucose challenge, similar to what is observed in patients with early type 2 Diabetes (Ohguni *et al.*, 1995).

CHAPTER THREE

Materials and Methods

3.0 This chapter describes the various methods and procedures used in this study

3.1Purchase and processing of soybeans

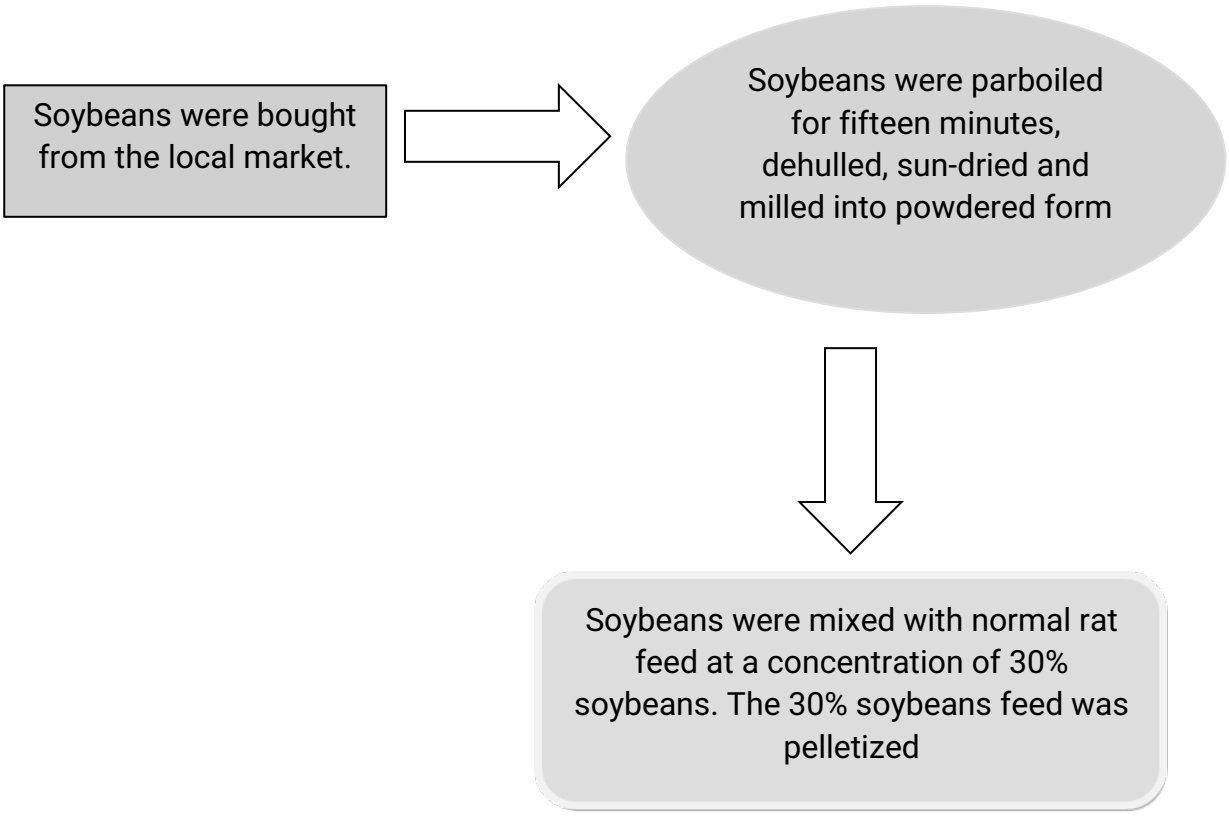


Fig 3.1 Purchase and processing of Soybeans

3.2 Purchase and processing of cassava

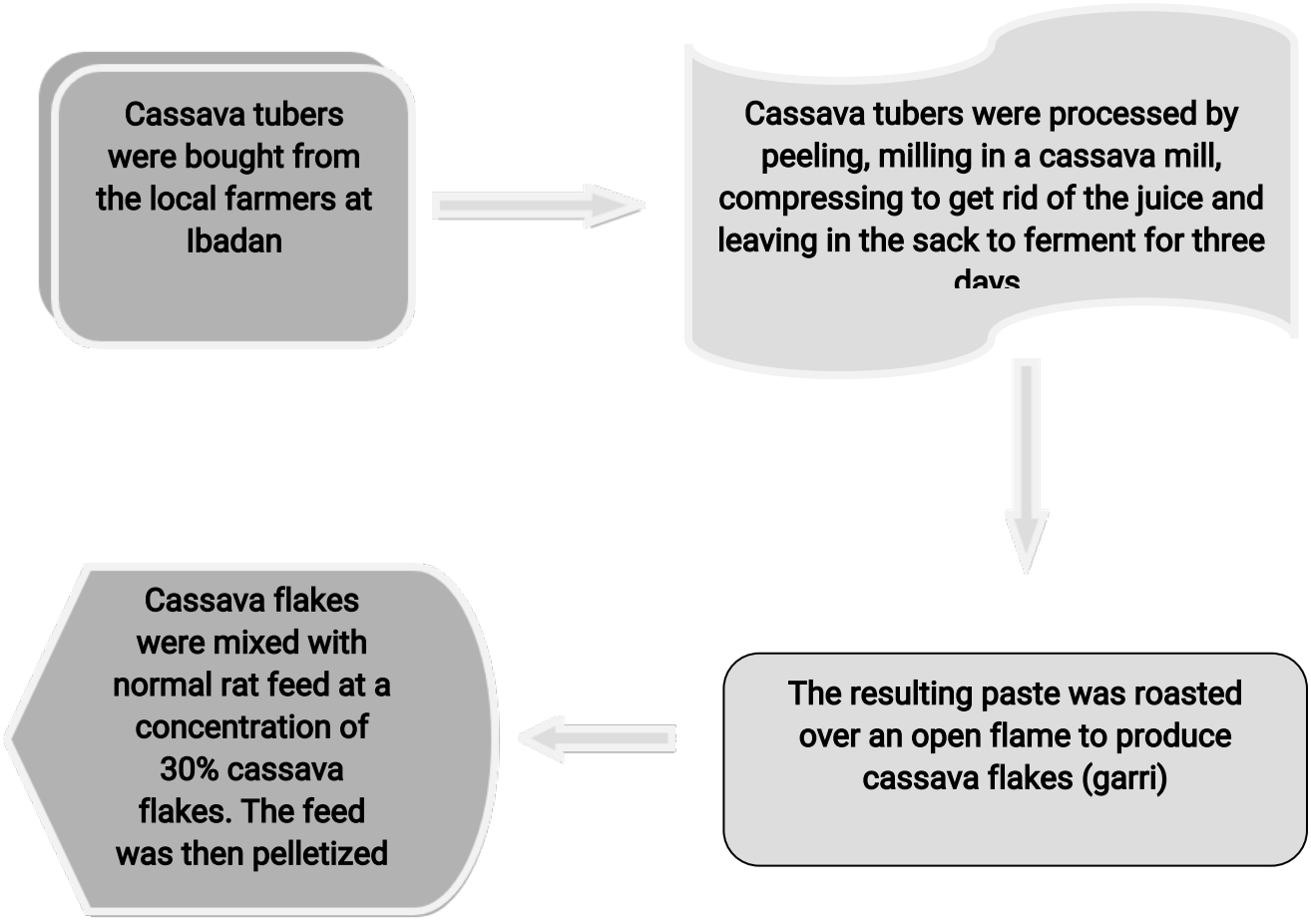


Figure 3.2 Purchase and processing of Cassava

3.3 Determination of Iodine and Caloric Content of Feeds

The standard rat feed, processed soybeans and cassava flakes were analysed to determine the following parametres;

1. **Determination of Iodine Content**

The method of A.O.A.C (1984) was used. 5g of sample was dissolved in approximately 100ml water. The pH was adjusted to 2.8 using 0.6% HCl. 30mg potassium iodide powder (KI) was added to convert all iodate present to elemental iodine.

The liberated iodine was titrated with 0.005N freshly prepared $Na_2S_2O_3$ (sodium thiosulphate solution) using 1% starch solution as the end point or equivalence point indicator. The titre obtained at this point was used to calculate iodine concentration in sample in mg/kg.

Iodine in mg/kg = titre value X 0.1058 X 100/ wt. of sample
Where weight of sample= 10g
Formula becomes: titre value X 10.58

N.B 1ml 0.005N $Na_2S_2O_3$ = 0.1058mgI

2. **Determination of Caloric content** (using a bomb calorimeter)

The apparatus used was the Gallenkamp Ballistic Bomb Calorimeter. Reagent used for calibration was Benzoic acid.

Determination: 0.25g of each sample depending on the bulkiness was weighed into the steel capsule. A 10cm cotton thread was attached to the thermocouple to touch the capsule. The bomb was closed and charged in with oxygen up to 30atm. The bomb was fixed up by depression to ignite switch to burn the sample in an excess of oxygen. The maximum temperature rise in the bomb was measured by thermocouple and galvanometer system. The rise in temperature with that obtained for 0.25gm of benzoic acid of each sample was determined by the following stepwise calculations:

Mass of Benzoic acid = W_1gm

Caloric Value of 1gm of Benzoic acid = 6.32Kcal/g

Heat released from Benzoic acid = 6.32 x W_1Kcal

Galvanometer deflection without sample = T_1

Galvanometer deflection of benzoic acid= T_2-T_1

Calibration constant = 6.32 x W_1 / T_2-T_1 =y

The standardising is repeated five times and average value calculated for y.

Mass of sample = 0.25gm

Galvanometer deflection with sample = T_3

Galvanometer deflection of sample = T_3-T_1

Heat released from sample = (T_3-T_1) y Kcal

Caloric value of sample = (T_3-T_1) y/0.25 Kcal/g

3.4 Experimental design

Sixty (60) adolescent male rats of the wistar strain were purchased at the animal house and kept in cages. The rats had access to food and water *ad libitum*.

The rats were grouped into three (3) broad groups (1, 2 and 3) with each having four (4) subgroups (A-D) of five (5) animals each.

TABLE 3.1 Animal grouping

GROUPS	SUBGROUPS	NUMBER OF ANIMALS PER SUBGROUP	NUMBER OF ANIMALS PER GROUP
Group 1 **Normal rat feed**	**Subgroup A** Non- diabetic rats	5	20
	Subgroup B Non-diabetic rats + iodine	5	
	Subgroup C Diabetic rats	5	
	Subgroup D Diabetic rats + iodine	5	
Group 2 **30% Soybeans feed**	**Subgroup A** Non- diabetic rats	5	20
	Subgroup B Non-diabetic rats + iodine	5	
	Subgroup C Diabetic rats	5	
	Subgroup D Diabetic rats + iodine	5	
Group 3 **30% Cassava feed**	**Subgroup A** Non- diabetic rats	5	

	Subgroup B Non-diabetic rats + iodine	5	20
	Subgroup C Diabetic rats	5	
	Subgroup D Diabetic rats + iodine	5	
Total Number of animals			60

The rats were kept for a total of 8 weeks before they were sacrificed.

3.5 Iodine supplementation

In groups B and D, iodine was added to the rat feeds which were fed to the rats *ad libitum* from the beginning to the end of the experiment (for 8 weeks). The iodine was mixed with their feeds at a concentration of 10mg/kg of feed; a method used by Kotyzováa *et al.,* (2005) for iodine supplementation. The requirement was determined by comparing the weight, iodine content and dry matter content of thyroid glands from rats supplemented with various levels of iodine.

3.6 Induction of Diabetes Mellitus

Diabetes was induced in subgroups C and D after 6 weeks with one dose of intraperitoneal administration of Alloxan monohydrate at a dose of 150 mg per kg rat body weight (Sikarwar and Patil, 2010) (Akinola *et al.*, 2012) after an overnight fast. The Alloxan was diluted in normal saline and administered within a few minutes. One hour after, the rats were given feed *ad libitum* and 5% dextrose. After 7 days, the fasting blood glucose of the rats was assessed with the use of Accu-check glucometre and strip. The rats with fasting blood glucose levels higher than 200 mg/dl were considered diabetic.

3.7 Oral Glucose Tolerance Test

At the end of the experiment, the rats were fasted overnight but had access to water.

A sterile razor was used to cut the tip of the tail of each rat to express a drop of blood from which the fasting blood sugar was determined using the glucose oxidase method with the aid of Accu-check glucometre and strips.

Each rat was administered 1.75g of glucose D per kg of body weight dissolved in distilled water at a concentration of 8.75g of glucose per 25mls of water orally and the time recorded. The blood sugar level was determined after 30 minutes of oral ingestion of glucose. This was repeated every 30 minutes for 2 hours and recorded (Arigi *et al.*, 2014).

3.8 Insulin Tolerance Test

After a six hour fast, the fasting blood glucose levels for each rat was measured with the aid of a glucometre strip and kit and Insulin 0.05 U/kg was injected intra-peritoneally, after which the blood glucose levels were investigated every thirty minutes for two hours (Jing Ai *et al.*, 2005) and recorded.

3.9 Determination of insulin resistance

The homeostatic model assessment of insulin resistance (HOMA_IR) developed by (Mathew *et al.*, 1985) was used to calculate insulin resistance using the following formulae:

HOMA-IR = fasting blood glucose (mmol/L or uIU/L) x fasting insulin (microU/L) ÷ 22.5

Healthy range = 1.0 (0.5-1.4)

>1.9 = Early insulin resistance

>2.9 = Significant insulin resistance

3.10 Determination of Body Weight of Experimental rats

Each rat was weighed with the use of an electronic weighing scale. The rats were weighed in grams and the body weights were recorded at the following periods;

i. Before the commencement of the experiment

ii. Six weeks after the commencement of the experiment (before induction of Diabetes Mellitus in the diabetic groups)

iii. At the end of the experiment

3.11 Collection of blood samples

Blood was collected into plain bottles from the orbital vein with the use of plain capillary tubes. The blood was centrifuged at 3,000rpm for 20 minutes after which the supernatant was separated with the use of a micropipette into separate plain bottles and frozen at -20°C. The serum samples were used for hormonal assays and determination of oxidative stress markers.

3.12 Sampling and measurement

Hormonal Assays

Thyroid Function Tests (T_4, T_3 and TSH)

ELISA (Enzyme-Linked Immuno-sorbent Assay) was used to determine the mean serum levels of T_3, T_4 and TSH, Thyroid peroxidase (TPO) and thyroglobulin (TG) antibodies using their respective ELISA kits. Blood specimens and serum were separated immediately and frozen at -20^{o}C. Prior to the assay, frozen samples were thawed completely and mixed well.

Determination of Serum T_4 levels

Reagent preparation

T_4 enzyme conjugate 1:11 was diluted with assay diluent in a suitable container. Wash buffer was prepared by adding the contents of the bottle (25ml, 20X) to 475ml of distilled water and stored at room temperature.

Assay procedure

Before proceeding with the assay, all reagents, serum references and controls were brought to room temperature (20-25^{o}C)

The microplates' wells were formatted for each serum reference, control and subject specimen to be assayed in duplicate. 25UL of the standards, control or specimen was pipetted into the assigned well50ul of the working T_4-enzyme conjugate solution was pipetted to all wells. 50ul of T_4-Antibody-Biotin was added to all wells.

The microplate was swirled gently for 20-30 seconds to mix the reagents. The microplate was covered and incubated for 60mins at room temperature. Liquid was removed from all wells. Wells were washed three times with 300ul of 1X wash buffer and blotted with absorbent paper towels

100ul of TMB substrate solution was added to all wells. The plate was covered an incubated at room temperature for 15mins. 50ul of stop solution was added to each well and mixed gently for 15-20 seconds.

Calculation of results

The absorbance was read on ELISA Reader for each well at 450nm within 15 minutes after adding the stop solution.

The standard curve was constructed and the absorbance for controls and each unknown sample were read from the curve and recorded.

Determination of Serum T_3 levels

Reagent preparation

T_3 enzyme conjugate was diluted 1:11 with assay diluents in a suitable container

Wash buffer was prepared by adding the contents of the bottle (25ml, 20X) to 475ml of distilled water and stored at room temperature.

Assay procedure

Before proceeding with the assay, all reagents, serum references and controls were brought to room temperature (20-25^{o}C).

The microplates' wells were formatted for each serum reference, control and subject specimen to be assayed in duplicate

50ul of the standards, control or specimens were pipette into the assigned well

50ul of the working T_3-enzyme conjugate solution was added to all wells

50ul of T_3-Antibody-Biotin solution was added to all wells

The microplate was swirled gently for 20-30 seconds to mix the reagents and covered and incubated for 60 minutes at room temperature.

Liquid was removed from all wells. The wells were washed three times with 300ul of 1X wash buffer and blotted with absorbent paper towels

100ul of TMB substrate solution was added to all wells. The plates were covered and incubated at room temperature for 15-20 seconds. 50ul of stop solution was then added to each well and gently mixed for 15-20 seconds.

Calculation of results

The absorbance was read on ELISA reader for each well at 450nm within 15minutes after adding the stop solution.

The standard curve was constructed and the absorbance for controls and each unknown sample were read from the curve and recorded.

Determination of Serum TSH levels

Reagent preparation

1X wash buffer was prepared by adding the contents of the bottle (25ml, 20X) to 475ml of distilled water and stored at room temperature.

Assay procedure

Prior to assay, reagents were allowed to stand at room temperature andgently mixed before use

The desired number of coated strips was placed into the holder. 50ul of TSH standards, control and subject specimen were pipetted into the designated wells

100ul of ready to use conjugate reagent was added to all wells and shook for 10-30 seconds

The plate was covered and incubated for 60 minutes at room temperature. Liquid was removed from all wells and wells were washed three times with 300ul of 1X wash buffer and blotted with absorbent paper towels. 100ul of TMB substrate was added to all wells and incubated for 15minutes at room temperature.

50ul of stop solution was added to all wells and shaken gently to mix the solution.

Calculation of results

The absorbance was read on ELISA reader for each well at 450nm within 15minutes after adding the stop solution.

The standard curve was constructed and the absorbance for controls and each unknown sample were read from the curve and recorded.

Determination of Thyroglobulin Antibody

Reagent preparation

1X wash buffer was prepared by adding the contents of the bottle (25ml, 20X) to 475ml of distilled water and stored at room temperature.

Assay procedure

Prior to assay, reagents were allowed to stand at room temperature and gently mixed before use. The desired number of coated strips were placed into the holder

Negative control, positive control and calibrator were ready to use.

1:2 dilution of test samples was prepared by adding 10ul of the sample to 200ul of sample dilluent and mixed well.

For the reagent blank, 100ul of diluted sera was dispensedin 1A well position. The holder was taped to remove air bubbles from the liquid and mixed well and incubated for 20minutes at room temperature.

The liquid was removed from all wells and wells were washed three times with 300ul of 1X wash buffer and blotted on absorbent paper towel.

100ul of enzyme conjugate was dispensed to each well and incubated for 20 minutes at room temperature.

Enzyme conjugate was removed from all wells and wells were washed three times with 300ul of 1X wash buffer and blotted with absorbance paper.

100ul of TMB substrate was added and incubated for 10minutes at room temperature

100ul of stop solution was added

Calculation of results

O.D was read at 450nm using ELISA reader within 15minutes

The Ab (Antibody) index of each was calculated by dividing the mean values of each sample by cut-off value (0.73). Positive index range (1.5-2.8), negative (0.1)

Determination of Thyroid Peroxidase Antibody (TPO IgG)

Reagent preparation

1X wash buffer was prepared by adding the contents of the bottle (25ml, 20X) to 475ml of distilled water and stored at room temperature.

Assay procedure

Prior to assay, reagents were allowed to stand at room temperature and gently mixed before use. The desired numbers of coated strips were placed into the holder.

Negative control, positive control and calibrator were ready to use.

1:2 dilution of test samples was prepared by adding 10ul of the sample to 200ul of sample dilluent and mixed well.

For the reagent blank, 100ul of diluted sera was dispensed in 1A well position. The holder was taped to remove air bubbles from the liquid and mixed well and incubated for 20minutes at room temperature.

The liquid was removed from all wells and wells were washed three times with 300ul of 1X wash buffer and blotted on absorbent paper towel

100ul of enzyme conjugate was dispensed to each well and incubated for 20 minutes at room temperature.

Enzyme conjugate was removed from all wells and wells were washed three times with 300ul of 1X wash buffer and blotted with absorbance paper

100ul of TMB substrate was added and incubated for 10minutes at room temperature

100ul of stop solution was added

O.D was read at 450nm using ELISA reader within 15minutes.

Calculation of results

The Ab (Antibody) index of each was calculated by dividing the mean values of each sample by cut-off value (0.6293). Positive index range (2.3-4.3), negative (0.1)

Determination of Serum Insulin levels

Reagent preparation

20X enzyme conjugate: 1X working dilution at 1:20 with assay dilluent was prepared as needed. The diluted conjugate was used the same day.

20X wash buffer concentrate: 1X wash buffer wash prepared by adding the contents of the bottle to 475ml of distilled water and stored at room temperature.

Assay procedure

Prior to the assay, the reagents were allowed to stand at room temperature. They were gently mixed before use.

The desired number of coated strips was placed in the holder. 25ul of insulin standards, control and subjects sera was pipette in appropriate wells. 100ul of working insulin enzyme conjugate was added to all wells and thoroughly mixed for 10 seconds.

It was incubated at room temperature for 60minutes. All liquid was removed from all wells and wells were washed three times with 300ul of 1X wash buffer and blotted on absorbent towels.

100ul of TMB substrate was added to all wells and incubated for 15minutes at room temperature. 50ul of stop solution was added to all wells and the plate was shaken to mix the solution. Absorbance was read on ELISA reader at 450 nm within 15minutes after adding the stopping solution.

Calculation of results

The standard curve was constructed and the absorbance for controls and each unknown sample were read from the curve and recorded.

Determination of Oxidative Stress Markers

Determination of Serum Lipid Peroxidation (Malondialdehyde-MDA) levels

Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation. This was carried out by the method of Varshney and Kale (1990).

Procedure

An aliquot of 0.4ml of the sample was mixed with 1.6ml of Tris-KCl buffer to which 0.5ml of 30% TCA was added. Then 0.5ml of 0.75% TBA was added and placed in a water bath for 45 minutes at 80 ^{o}C. This was then cooled in ice and centrifuged at 3000rpm for 15 minutes. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532nm. The MDA level was calculated according to the method of Adam-Vizi and SEregi (1982). Lipid peroxidation in units/mg protein or gram tissue was computed with a molar extinction coefficient of $1.56X10^{5}$ M^{-1} Cm^{-1}.

Determination of Serum Superoxide Dismutase (SOD) Activity

The level of SOD activity was determined by the method of Misra and Fridovich (1972).

Protocol

1ml of sample was diluted in 9ml of distilled water to make a 1 in 10 dilution. An aliquot of 0.2 ml of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds.

Calculation

Increase in absorbance per minute = A3-A0 2.5

Where A0 =absorbance after 30 seconds

A3 = absorbance after 150 seconds

$$\% \text{ inhibition} = 100 - \frac{\text{increase in absorbance for substrate} \times 100}{\text{increase in absorbance for blank}}$$

Determination of Total Serum Nitrite (NO)

Nitrite determination was done using the method described by Ignarro *et al.,* (1987). The procedure is based on the chemical reaction which uses sulfanilamide and naphthylethylenediamine dihydrochlorate (NED) and under acidic condition. Sulfanilamide and NED compete for nitrite in the Griess reaction.

Procedure

Greiss reagent was prepared by mixing equal volume of 0.1% NED and 1% sulfanilic acid in 5% phosphoric acid. 300ul of sample was added to 2.6ml of distilled water and 100ul of greiss reagent was added. The mixture was incubated at room temperature and protected from light for 15minutes to allow for colour development. The absorbance was measured within 30minutes at 550nm.

3.13 Statistical Analysis

Data was analysed using ANOVA and students 'T test' for unpaired groups was used to calculate the level of significance.

The P values less than or equal to 0.05 (95% confidence) were taken as significant.

* Significantly different from control ≤ 0.05
** Significantly different from control ≤ 0.01
***Significantly different from control ≤ 0.001

3.14 Ethical considerations

Wistar rats were purchased from the central animal house, faculty of Basic Medical Sciences. They were housed in well-ventilated solid-bottom polyprolene cages with wire mesh covers. The rats were allowed to acclimatize for two weeks and were fed with standard rat feed and water *ad libitum*. There were physically examined to check their health status. This research was approved by the animal care and use research ethics committee (ACUREC), University of Ibadan.

CHAPTER FOUR

Results

4.0 The results of all analysis carried out in all the studies are presented in this chapter

4.1 Results of mean iodine and caloric contents of normal rat feed, soybeans and cassava

The mean iodine and caloric contents of normal rat feed, soybeans and cassava are presented in Table 4.1 in the next page. From the table, the following were observed;

The iodine content in soybeans was significantly higher than the iodine content in normal rat feed and cassava.

The iodine content in normal rat feed was significantly higher than the iodine content in cassava and lower than that of soybeans.

The gross energy content in normal rat feed was significantly higher than that of soybeans and cassava.

The gross energy content in cassava was significantly lower than that of soybeans and normal rat feed.

Table 4.1: Mean Iodine and Caloric Contents of normal rat feed, soybeans and cassava.

Sample	Iodine (mg/kg)	Gross energy(Kcal/g)
Normal Rat Feed	5.68±0.009	3.97±0.0014
Glycine max (soybeans)	7.27±0.018*	3.45±0.0014
Manihot esculenta (Cassava)	3.73±0.014*	2.93±0.0014*

Data are expressed as mean ±SEM. Comparisons were made using one way ANOVA and students 'T test' for unpaired groups. The symbol * denotes $p \leq 0.05$ when compared to the control group (Normal rat feed).

The results for mean iodine and caloric content of Rat feeds are presented in table 4.2 in the next page. From the table, the following were observed;
The iodine content in normal rat feed was significantly lower than in the 30% soybeans feed. This indicates that the experimental rats that consumed normal rat feed (group 1) had lower iodine intake per gram of feed than the experimental rats that consumed 30% soybeans (group 2).

The iodine content in 30% soybean feed was significantly higher than the iodine content in 30% cassava feed. This implies that the experimental rats fed with 30% soybeans (group 2) had a higher iodine intake per gram of feed than the experimental rats fed with 30% cassava (group 3).

There was no significant difference between the iodine content of normal rat feed in comparison to 30% cassava feed. This shows that the experimental rats fed with 30% cassava (group 3) consumed the same quantity of iodine per gram of feed as the rats in group 1 (normal rat feed).

There was no significant difference between the gross energy content (Kcal/g) in normal rat feed in comparison to that of 30% soybeans feed and 30% cassava feed. This indicates that the experimental rats in group 1(normal rat feed group), group 2 (30% soybeans feed) and group 3 (30% cassava feed) consumed the same amount of calories in each gram of feed.

Table 4.2 Mean Iodine and Caloric Content of Rat Feeds

Sample	Iodine content(mg/kg)	Gross energy(kcal/g)
Normal Rat Feed	5.68±0.009	3.97±0.0014
Rat feed with 10mg/kg iodine added	15.68±0.009	3.97±0.0014
Rat feed with 30% *Glycine max*	6.15±0.018*	3.81±0.0014
Rat feed with 30% *Glycine max* and 10mg/kg iodine added	16.15±0.018*	3.81±0.0014
Rat feed with 30% *Manihot esculenta*	5.09±0.014	3.66±0.0014
Rat feed with 30% *Manihot esculenta* and 10mg/kg iodine added	15.09±0.014	3.66±0.0014

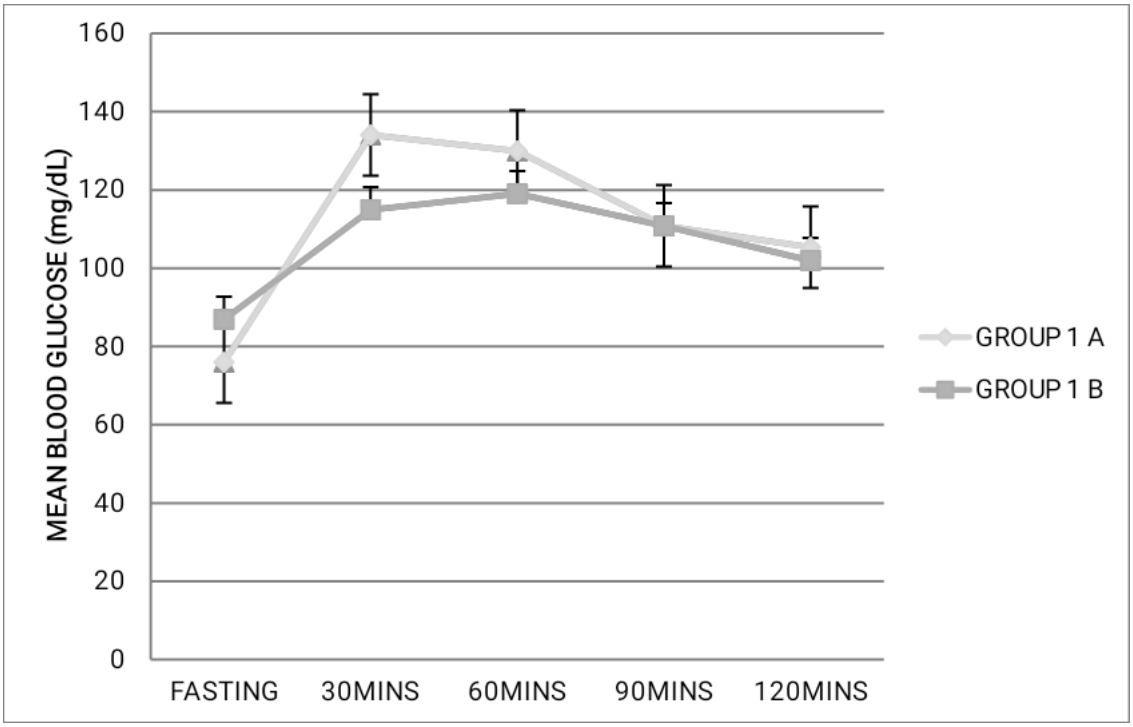
Data are expressed as Mean ±SEM. Comparisons were made using one way ANOVA and students 'T test' for unpaired groups. The symbol * denotes $p \leq 0.05$ when compared to the control group (Normal rat feed).

4.2 Results of Oral Glucose Tolerance Tests

Results for oral glucose tolerance test in groups 1A and 1B are presented in figure 4.1 in the next page. From the figure, the following were observed;

The mean fasting blood glucose level of group 1A was significantly lower than group 1B which indicates that iodine intake caused a significant increase in mean fasting blood glucose levels ($p \leq 0.05$) in non-diabetic rats that consumed normal rat feed.

There was increase in blood glucose level of group 1A in comparison to group 1B after 30 minutes of glucose intake. There was no significant difference between the mean blood glucose levels of group 1A and 1B after 60 minutes, 90 minutes and 120 minutes of glucose ingestion. This is an indication that iodine intake caused lower blood glucose levels 30 minutes after intake of glucose during the oral glucose tolerance test and there was no significant change in mean blood glucose level after 60 minutes of glucose intake in non-diabetic rats that were fed with normal rat feed.



p ≤ 0.05

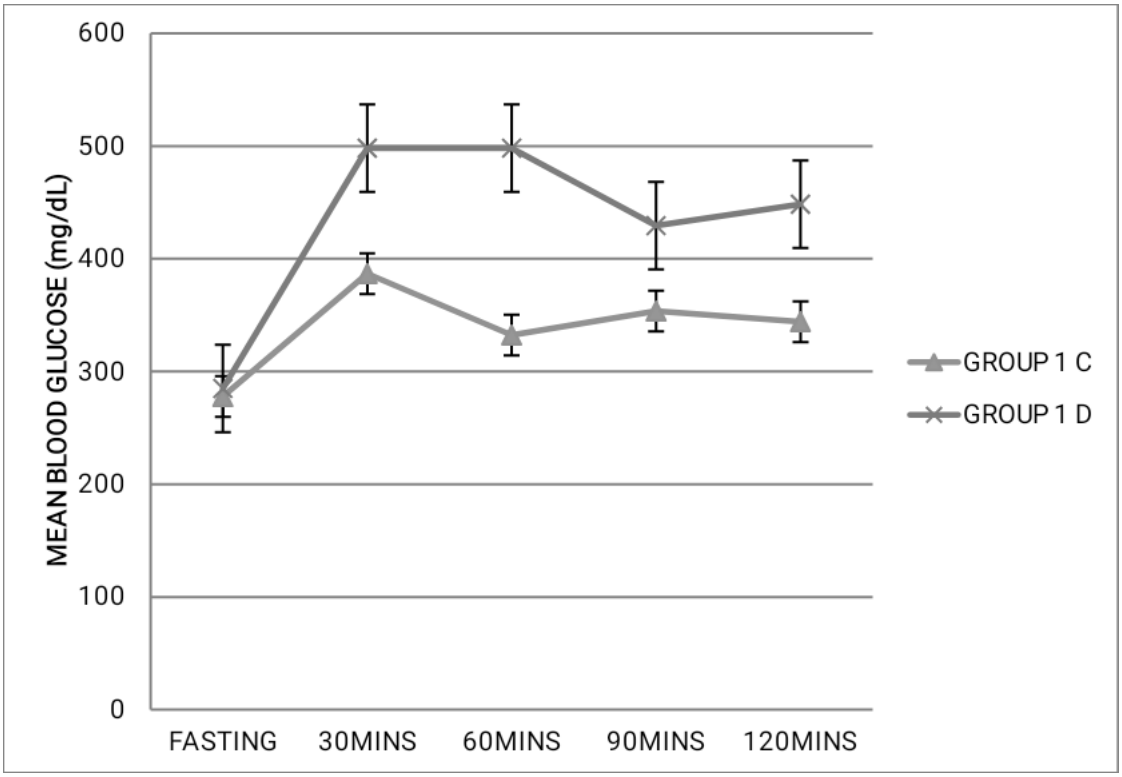
Figure 4.1 Oral Glucose Tolerance Test for Groups 1A and 1B.

Group 1A= Non-diabetic rats with normal rat feed, Group 1B=Non-diabetic rats with normal rat feed + iodine (10 mg/kg of feed). Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for oral glucose tolerance test in groups 1C and 1D are presented in figure 4.2 in the next page. From the figure, the following were observed

There was no significant difference between the mean fasting blood glucose levels of groups 1C and 1D which is an indication that iodine intake had no significant effect on the mean fasting blood glucose levels in diabetic experimental rats fed with normal rat feed.

The mean blood glucose levels in group 1C was significantly lower in comparison to group 1D after 30 minutes, 60 minutes, 90 minutes and 120 minutes of glucose ingestion. This is an indication that iodine intake at 10 mg/kg of feed caused elevated mean blood glucose levels in diabetic rats fed with normal rat feed throughout the oral glucose tolerance test.



p ≤ 0.05

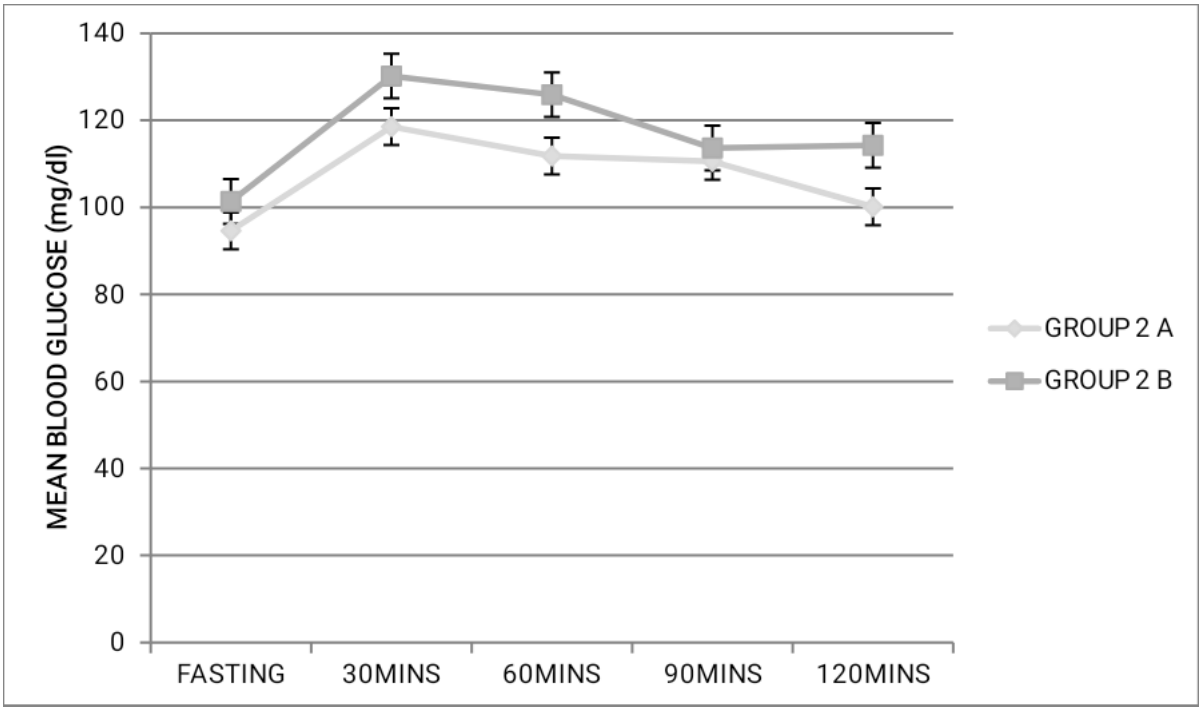
Figure 4.2 Oral Glucose Tolerance Test for Groups 1C and 1D

Group 1 C= Diabetic rats + normal rat feed, Group 1 D = Diabetic rats + normal rat feed + Iodine (10 mg/kg of feed). Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for oral glucose tolerance of groups 2A and 2B are presented in figure 4.3 in the next page. From the figure, the following were observed

There was no significant difference between the mean fasting blood glucose level of groups 2A and 2B which shows that iodine intake had no significant effect on the mean fasting blood glucose level of experimental rats that consumed 30% soybeans.

The mean blood glucose level of group 2A was significantly lower than group 2B after 30 minutes and 60 minutes of oral ingestion of glucose. There was no significant difference in the mean blood glucose level in group 2A and 2B after 90 minutes. The mean blood glucose level in group A was significantly lower than in group B after 120 minutes. This is an indication that iodine intake elevated blood glucose levels in rats that consumed 30% soybeans in the first hour and after two hours of glucose tolerance test.



p ≤ 0.05

Figure 4.3 Oral Glucose Tolerance Test Results for Groups 2A and 2B

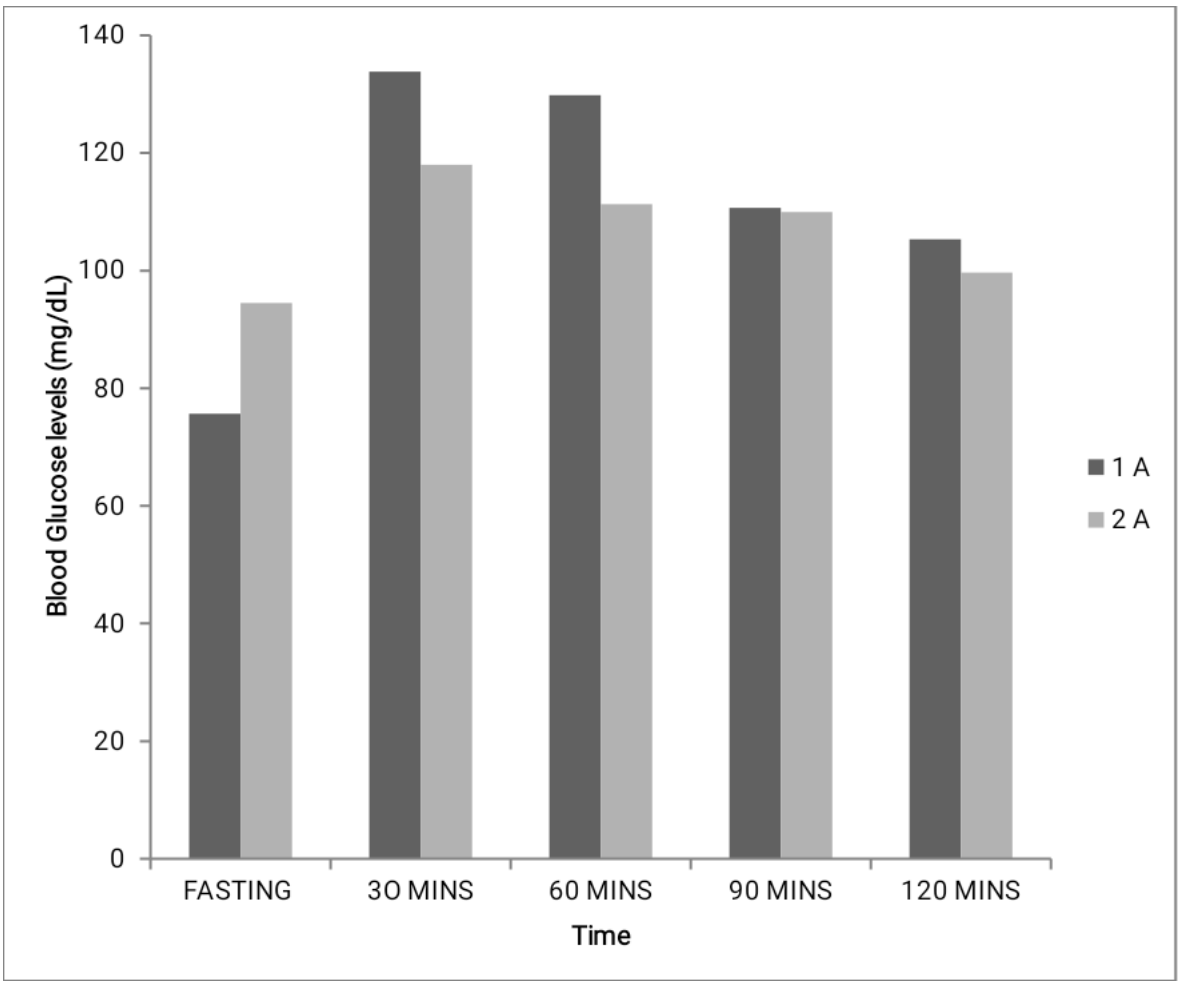
Group 2A = Non-diabetic rats fed with 30% Soybeans, Group 2B = Non-diabetic rats fed with 30% Soybeans + Iodine (30 mg/kg of feed). Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students' 'T test' for unpaired groups.

The results of oral glucose tolerance test for groups 1A and 2A are presented in figure 4.4 in the next page. From the figure, the following were observed;

The mean fasting blood glucose level of group 1A was significantly lower *($p \leq 0.05$)* in comparison to group 2A which is an indication that consumption of 30% soybeans caused elevated mean fasting blood glucose levels in non-diabetic rats in comparison to non-diabetic rats that consumed normal rat feed.

The mean blood glucose level of group 1A was significantly higher *($p \leq 0.05$)* than group 2A after 30 minutes of glucose ingestion. This shows that intake of 30% soybeans in non-diabetic rats significantly lowered blood glucose levels 30 minutes after glucose ingestion in comparison to the rats that consumed normal rat feed.

There was no significant difference in the mean blood glucose levels of groups 1A and 2A after 60 minutes, 90 minutes and 120 minutes of glucose ingestion during the oral glucose tolerance test. This is an indication that 30% soybean intake did not significantly affect glucose homeostasis after 60 minutes of glucose intake in comparison to the rats fed with normal rat feed.



p ≤ 0.05

Figure 4.4 Oral Glucose Tolerance Test Result for Groups 1A and 2A

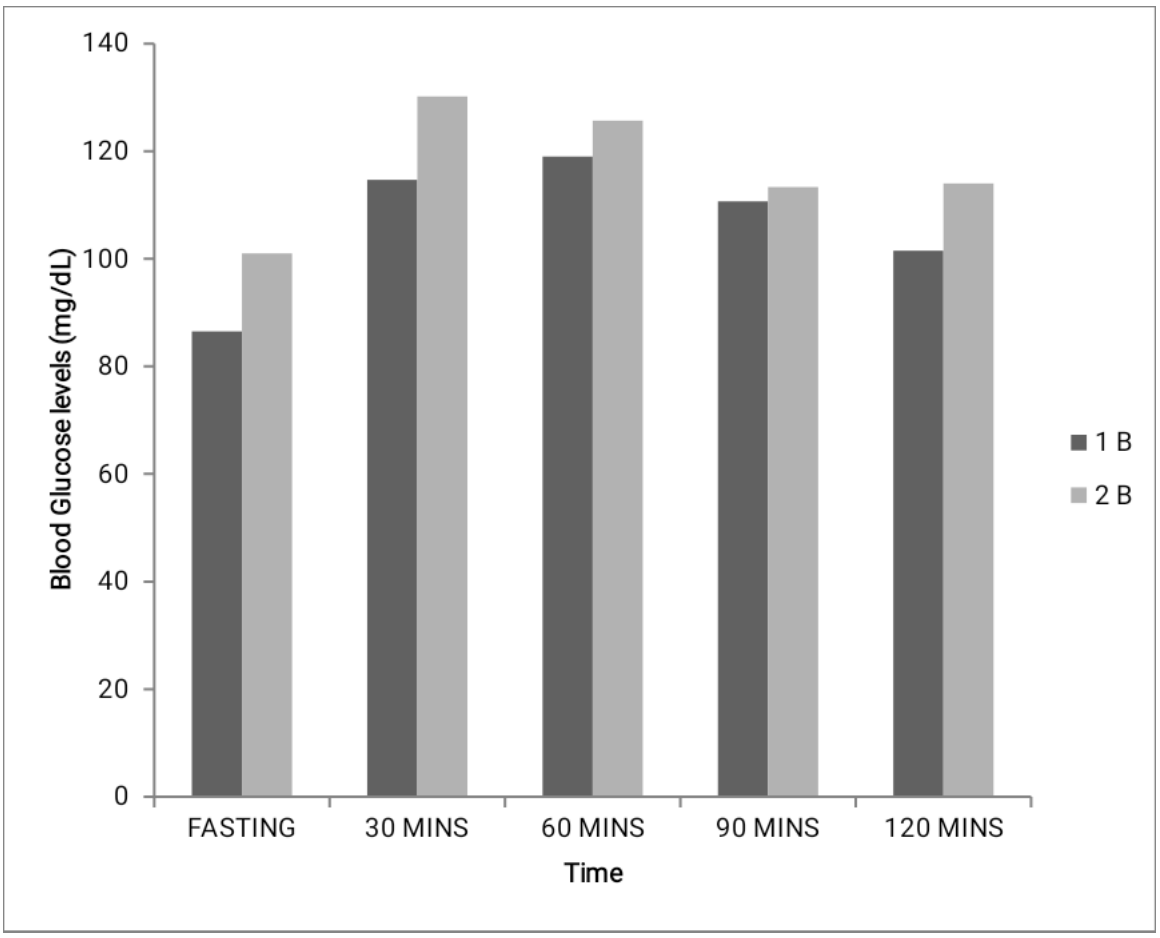
Group 1A=Non-diabetic rats with normal rat feed, Group 2A= Non-diabetic rats fed with 30% Soybeans. Data are expressed in mean ±SEM (n=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for oral glucose tolerance test for groups 1B and 2B are presented in figure 4.5 in the next page. From the figure, the following were observed;

The mean fasting blood glucose level of group 1B was significantly lower than that of Group 2B which shows that in non-diabetic rats, concurrent intake of 30% soybeans and iodine significantly increased mean fasting blood glucose levels in comparison with non-diabetic rats that consumed normal rat feed and iodine.

There was no significant difference in the mean blood glucose levels of groups 1B and 2B after 30 minutes, 60 minutes and 90 minutes of glucose ingestion. This is an indication that in comparison with non-diabetic rats that consumed normal rat feed and iodine, concurrent intake of 30% soybeans had no significant effect on glucose homeostasis for the first 90 minutes after intake of glucose.

The mean blood glucose level in group 1B was significantly lower when compared to group 2B 120 minutes after oral glucose ingestion. This shows that consumption of 30% soybeans and iodine elevated the mean blood glucose level in non-diabetic rats two hours after intake of glucose in comparison with those fed with normal rat feed and iodine.



p ≤ 0.05

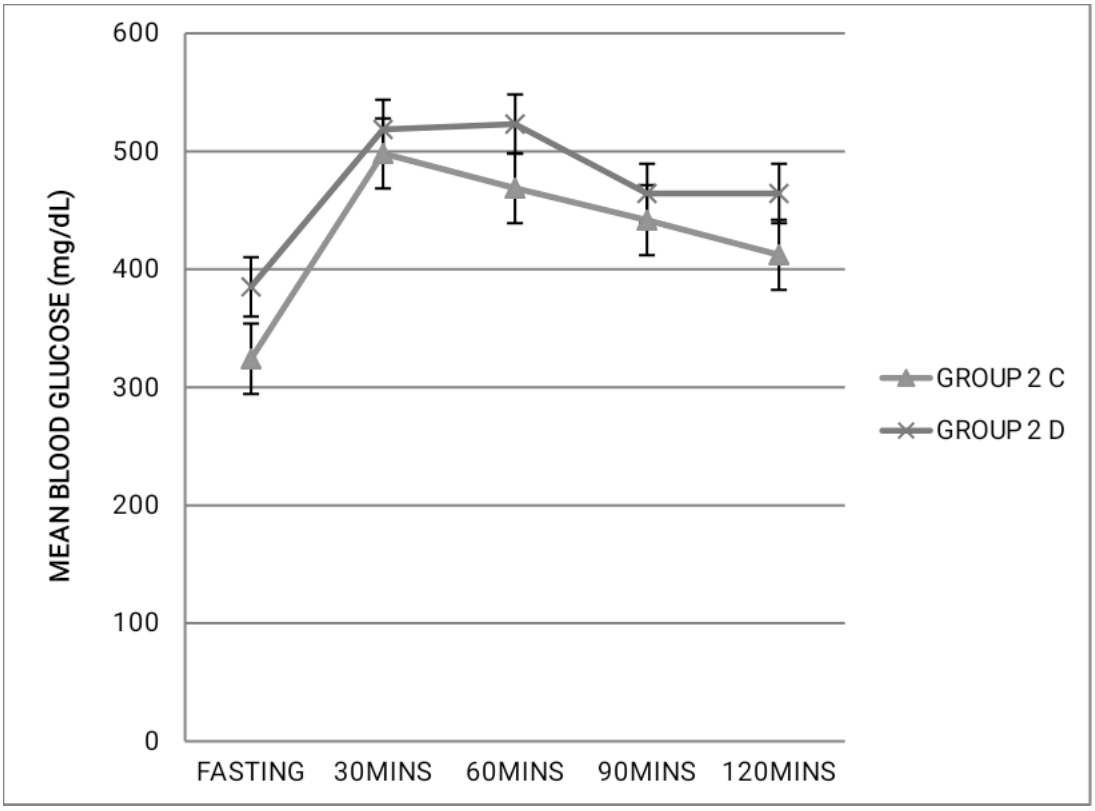
Figure 4.5 Oral Glucose Tolerance Test Result for Groups 1B and 2B

Group 1B= Non-diabetic rats with normal rat feed + Iodine (10 mg/kg of feed), Group 2B= Non-diabetic rats fed with 30% Soybeans + Iodine (10 mg/kg of feed). Data are expressed in mean ±SEM (n=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for oral glucose tolerance test in groups 2C and 2D are presented in figure 4.6 in the next page. From the figure, the following were observed;

The mean fasting blood glucose level in group 2C was significantly lower than in group 2D which shows that concurrent intake of iodine and 30% soybeans increased mean fasting blood glucose levels in diabetic rats.

The mean blood glucose levels after of group 2C was significantly lower than group 2D after 30 minutes and 60 minutes of glucose intake. There was no significant difference between the mean blood glucose levels of groups 2C and 2D 90 minutes and 120 minutes after ingestion of glucose. This is an indication that in comparison to the diabetic rats that took only 30% soybeans, concurrent iodine and 30% soybeans intake by diabetic rats significantly elevated blood glucose levels for the first 60 minutes of glucose tolerance test but had no significant effect on mean blood glucose levels after 90 minutes of glucose tolerance test.



p ≤ 0.05

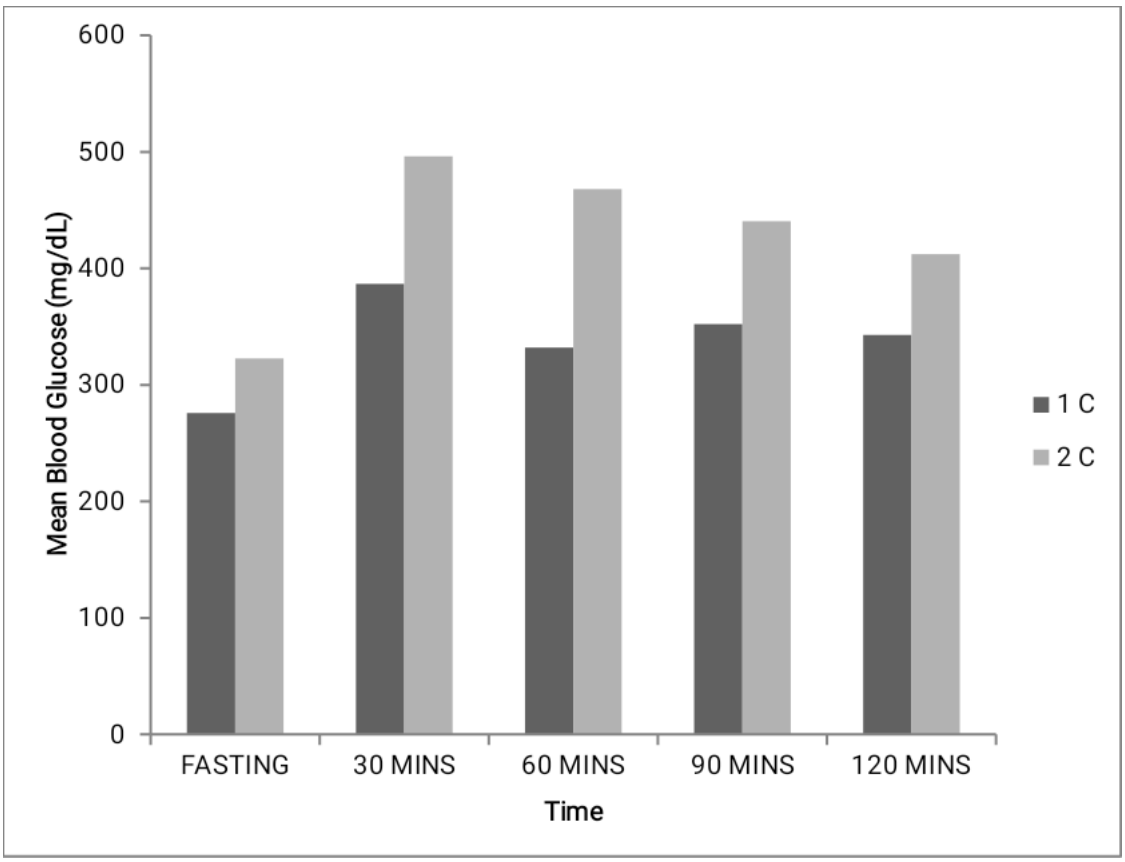
Figure 4.6 Oral Glucose Tolerance Test Results for Group 2C and 2D.

Group 2C= Diabetic rats fed with 30% soybeans feed, Group 2D= Diabetic rats fed with 30% soybeans feed + Iodine (10 mg/kg of feed). Data are expressed in mean ±SEM (n=5). Comparisons were made using one way ANOVA and students' 'T test' for unpaired groups.

The results for oral glucose tolerance test in groups 1C and 2C are presented in table 4.7 in the next page. From the figure, the following were observed;

The mean fasting blood glucose level in group 1C was significantly lower in comparison to group 2C which is an indication that 30% soybeans consumption significantly increased fasting blood glucose level in diabetic rats in comparison to normal rat feed intake.

The mean glucose levels in group 1C was significantly lower than in group 2C after 30 minutes, 60 minutes, 90 minutes and 120 minutes of glucose ingestion during the glucose tolerance test. This shows that intake of 30% soybeans significantly elevated blood glucose levels in diabetic rats throughout the glucose tolerance test in comparison to consumption of normal rat feed.



p ≤ 0.05

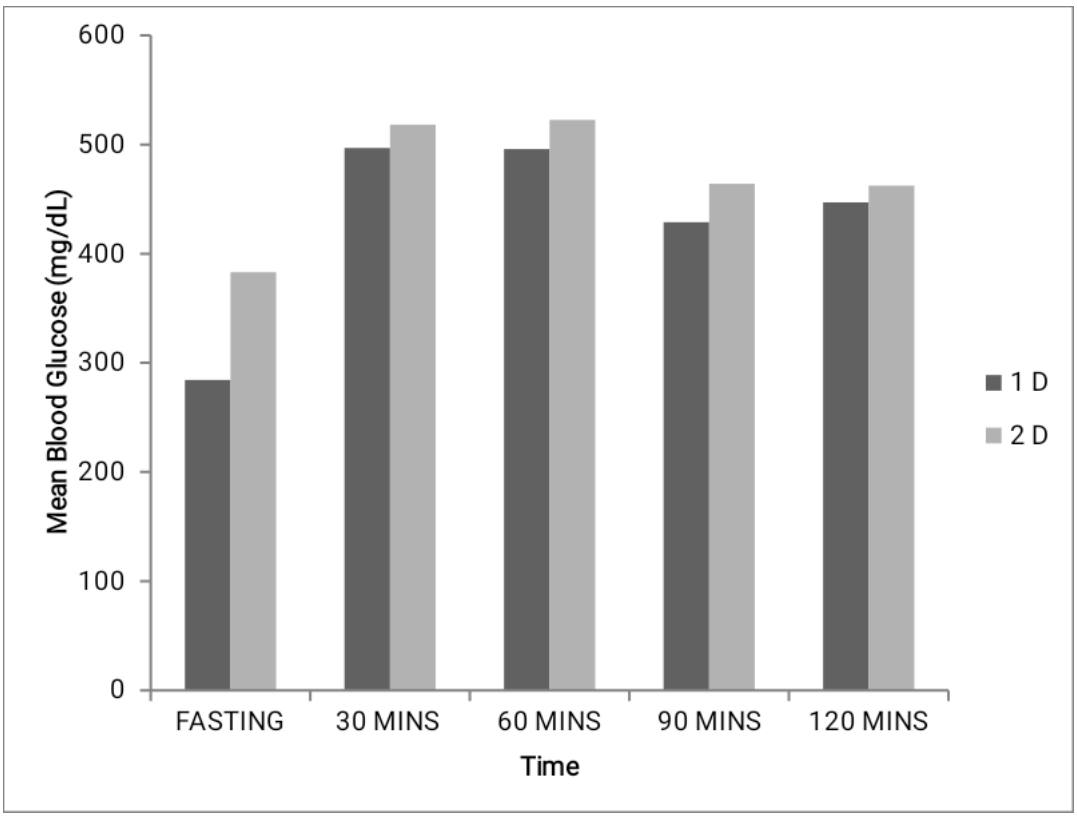
Figure 4.7 Oral Glucose Tolerance Test Results for Groups 1C and 2C

Group 1C= Diabetic rats fed with normal rat feed, Group 2C= Diabetic rats fed with 30% soybeans feed. Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA followed and students 'T test' for unpaired groups.

The results for oral glucose tolerance tests in groups 1D and 2D are presented in figure 4.8 in the next page. From the figure, the following were observed

The mean fasting blood glucose level of group 1D was significantly lower than group 2D which is an indication that concurrent consumption of iodine and 30% soybeans caused elevated mean fasting blood glucose level in diabetic rats in comparison to consumption of iodine and normal rat feed.

There was no significant difference in the mean glucose levels of groups 1D and 2D after 30 minutes, 60 minutes, 90 minutes and 120 minutes of glucose ingestion. This is an indication that concurrent intake of 30% soybeans and iodine did not alter blood glucose homeostasis in diabetic rats in comparison to the rats that consumed normal rat feed and iodine.



p ≤ 0.05

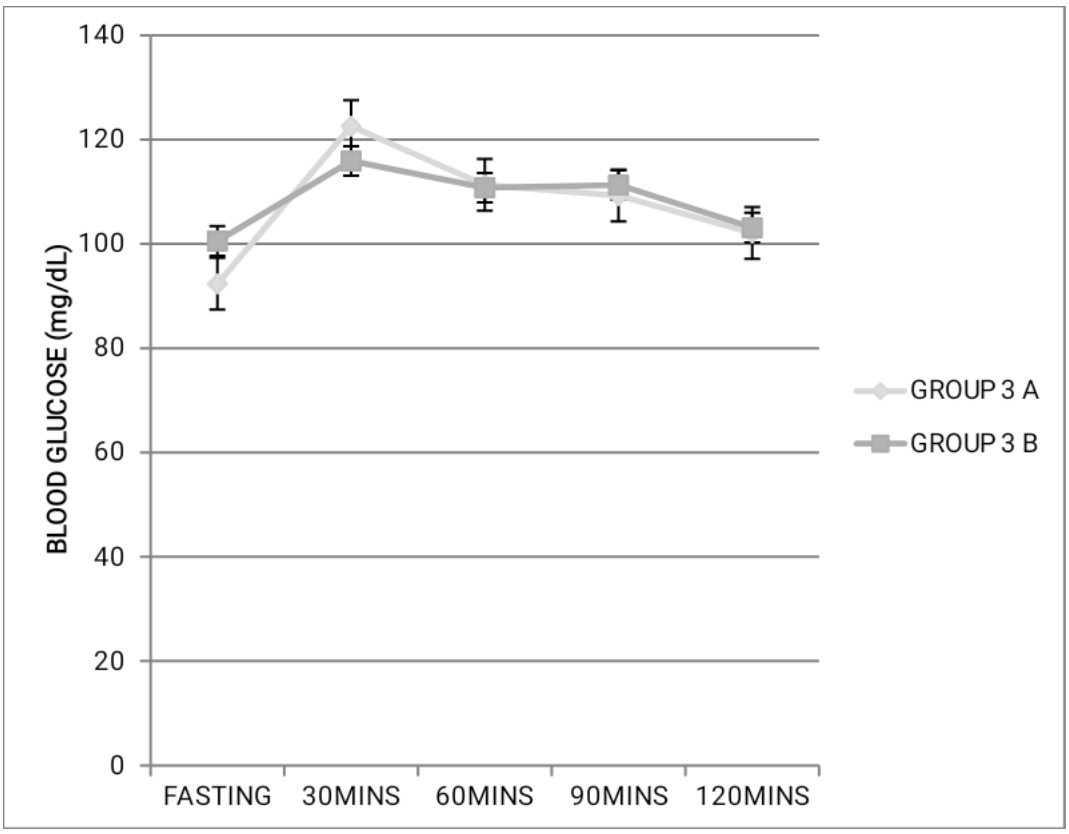
Figure 4.8 Oral Glucose Tolerance Test Results for Groups 1D and 2D

Group 1D = Diabetic rats fed with normal rat feed + Iodine (10 mg/kg of feed), Group 2D = Diabetic rats fed with 30% soybeans feed + Iodine (10 mg/kg of feed). Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for oral glucose tolerance tests are presented in figure 4.9 in the next page. From the figure, the following were observed

The mean fasting blood glucose level of group 3A was significantly lower than group 3B which implies that concurrent consumption of iodine and 30% cassava significantly elevated mean fasting blood glucose level in non-diabetic rats in comparison to intake of 30% cassava intake alone.

There was no significant difference in the mean blood glucose levels in group 3A and 3B after 30 minutes, 60 minutes, 90 minutes and 120 minutes of glucose ingestion. This is an indication that concurrent intake of iodine and cassava did not significantly alter glucose homeostasis in non-diabetic rats during the oral glucose tolerance test when compared to the intake of 30% cassava only.



p ≤ 0.05

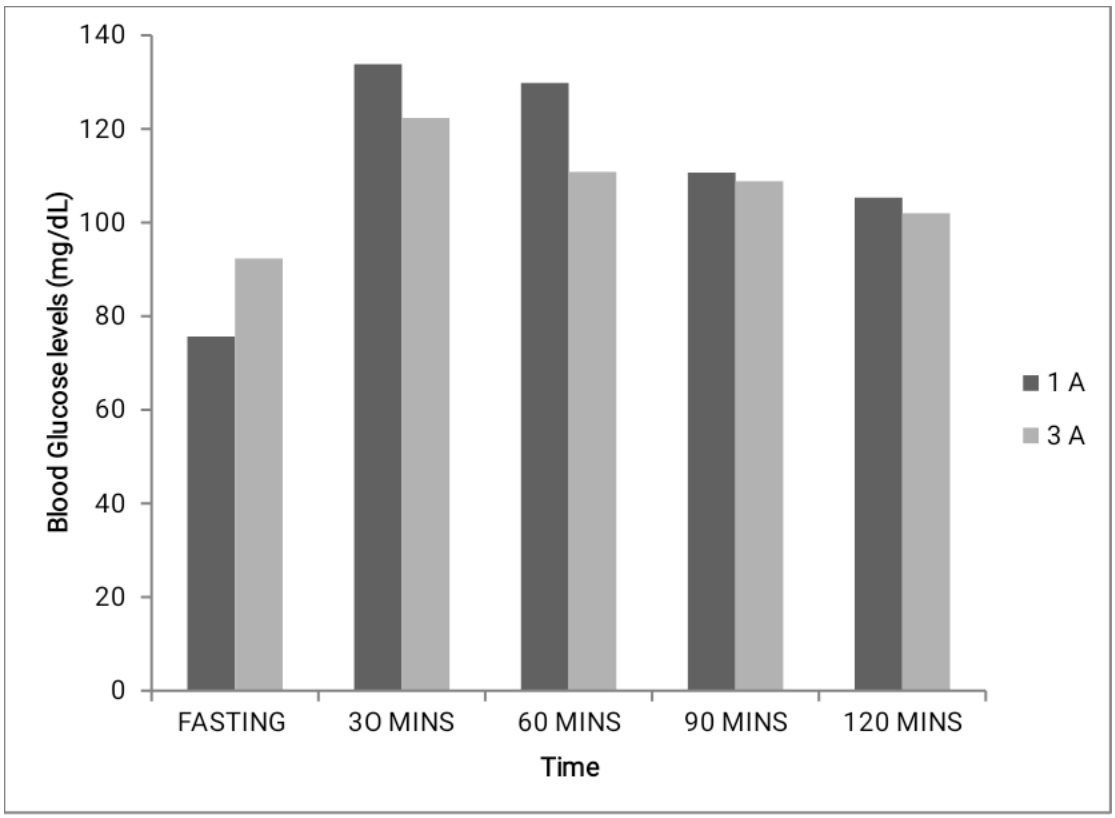
Figure 4.9 Oral Glucose Tolerance Test Results for Group 3A and 3B

Group 3A = Non-diabetic rats fed with 30% cassava feed, Group 3B = Non-diabetic rats fed with cassava feed + Iodine (10 mg/kg of feed). Data are expressed in mean ±SEM (n=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for oral glucose tolerance tests in groups 1A and 3A are presented in figure 4.10 in the next page. From the figure, the following were observed

The mean fasting blood glucose level of group 1A was significantly lower than that of Group 3A which is an indication that intake of 30% cassava significantly elevated mean fasting blood glucose level in non-diabetic rats when compared with intake of normal rat feed.

There was no significant difference in the mean blood glucose levels of groups 1A and 3A after 30 minutes, 60 minutes, 90 minutes and 120 minutes of glucose ingestion. This implies that intake of 30% cassava did not significantly affect mean blood glucose levels during the oral glucose tolerance test in comparison with intake of normal rat feed.



p ≤ 0.05

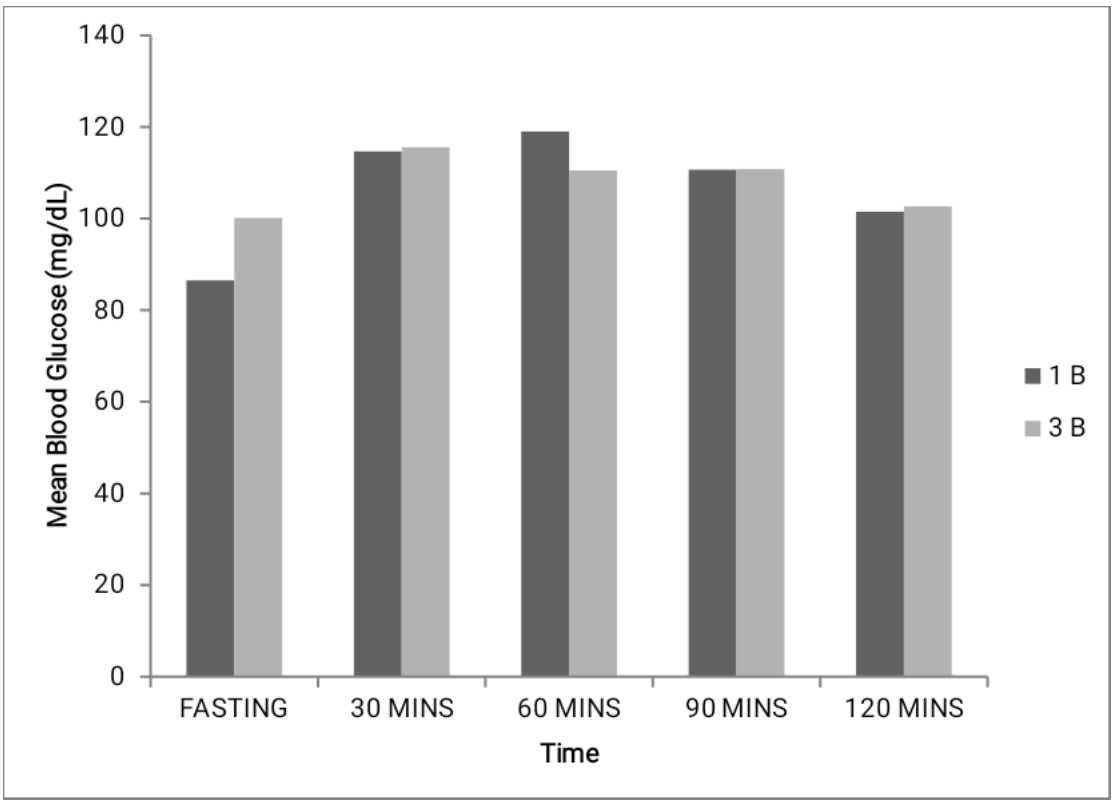
Figure 4.10 Oral Glucose Tolerance Test Result for Groups 1A and 3A

Group 1A= Non-diabetic rats with normal rat feed, Group 3A= Non-diabetic rats fed with 30% Cassava. Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for oral glucose tolerance tests in groups 1B and 3B are presented in figure 4.11 in the next page. From the figure, the following were observed;

The mean fasting blood glucose level of group 1B was significantly lower than group 3B which implies that the concurrent intake of iodine and cassava significantly increased mean fasting blood glucose level in non-diabetic rats in comparison to intake of normal rat feed and iodine.

There was no significant difference between the mean blood glucose levels of group 1B and 3B after 30 minutes, 60 minutes, 90 minutes and 120 minutes of glucose intake. This is an indication that concurrent iodine and 30% cassava intake did not significantly affect glucose homeostasis during the oral glucose tolerance test in non-diabetic rats in comparison to intake of iodine and normal rat feed.



p ≤ 0.05

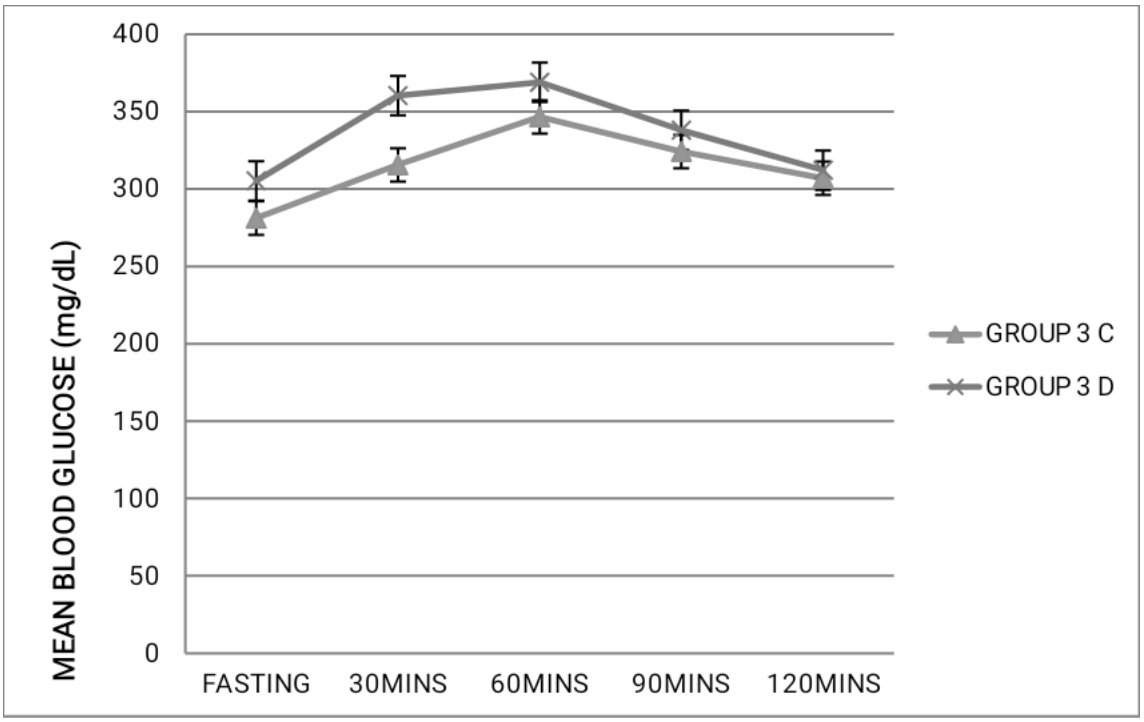
Figure 4.11 Oral Glucose Tolerance Test Result for Groups 1B and 3B

Group 1B= Non-diabetic rats with normal rat feed + Iodine (10 mg/kg of feed), Group 3B= Non-diabetic rats fed with 30% Cassava + Iodine (10 mg/kg of feed). Data are expressed in mean ±SEM (n=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for oral glucose tolerance tests in groups 3C and 3D are presented in figure 4.12 in the next page. From the figure, the following were observed

The mean fasting blood glucose level in group 3C was significantly lower than group 3D which implies that concurrent intake of iodine and 30% cassava caused elevated mean fasting blood glucose level in diabetic rats in comparison to intake of only 30% cassava.

There was no significant difference in the mean blood glucose levels of group 3C and 3D after 30 minutes, 60 minutes, 90 minutes and 120 minutes of glucose ingestion. This shows that concurrent iodine and 30% cassava intake did not significantly affect glucose homeostasis during the oral glucose tolerance test in diabetic rats in comparison with intake of only 30% cassava feed.



p ≤ 0.05

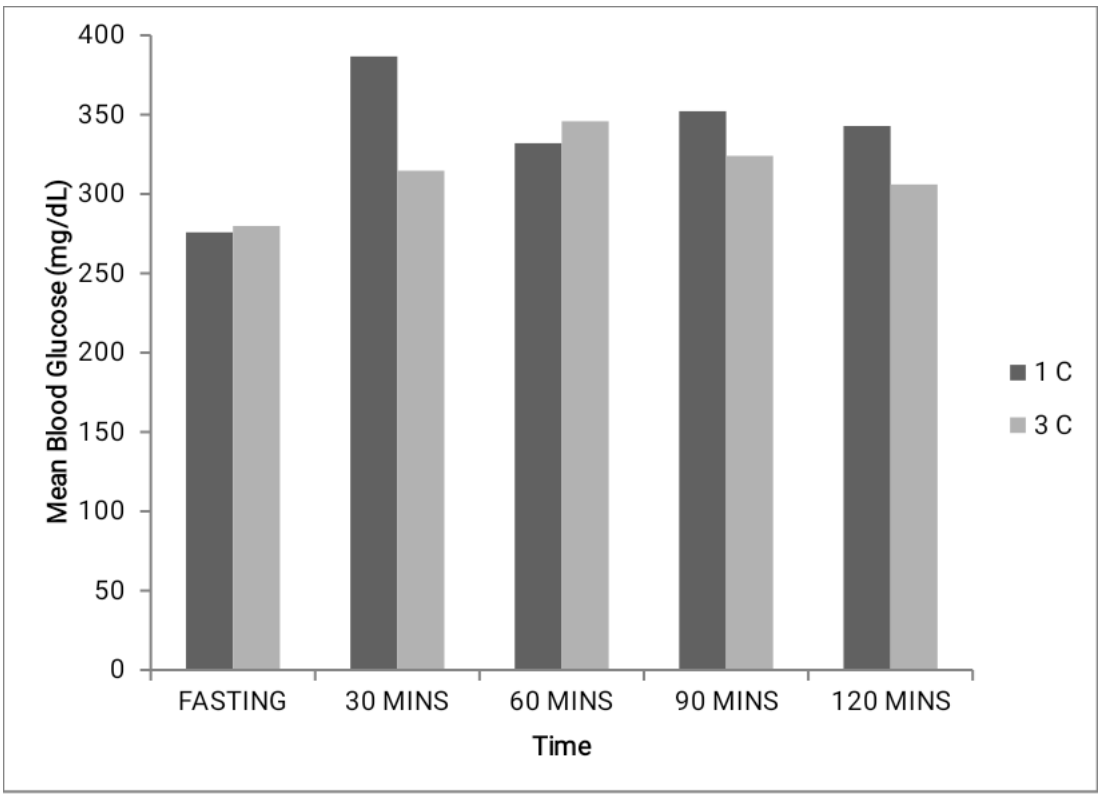
Figure 4.12 Oral Glucose Tolerance Test Result for Groups 3C and 3D

Group 3C= Diabetic rats with 30% cassava feed, Group 3D= Diabetic rats fed with 30% Cassava feed + Iodine (10 mg/kg of feed). Data are expressed in mean ±SEM (n=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for oral glucose tolerance tests in groups 1C and 3C are presented in figure 4.13 in the next page. From the figure, the following were observed;

There was no significant difference between the mean fasting blood glucose levels of group1C and group 3C which implies that intake of 30% cassava did not significantly alter mean fasting blood glucose level in diabetic rats in comparison to intake of normal rat feed.

The mean blood glucose level in group 1C was significantly higher than group 3C after 30 minutes of glucose ingestion. There was no significant difference in mean blood glucose levels of group 1C and group 3C after 60 minutes of glucose ingestion. The mean blood glucose level of group 1C was significantly higher than group 3C 90 minutes and 120 minutes after glucose ingestion. This is an indication that when compared with diabetic rats that consumed normal rat feed, diabetic rats fed with 30% cassava had lowered mean blood glucose levels 30 minutes, 90 minutes and 120 minutes after glucose intake during the oral glucose tolerance test.



p ≤ 0.05

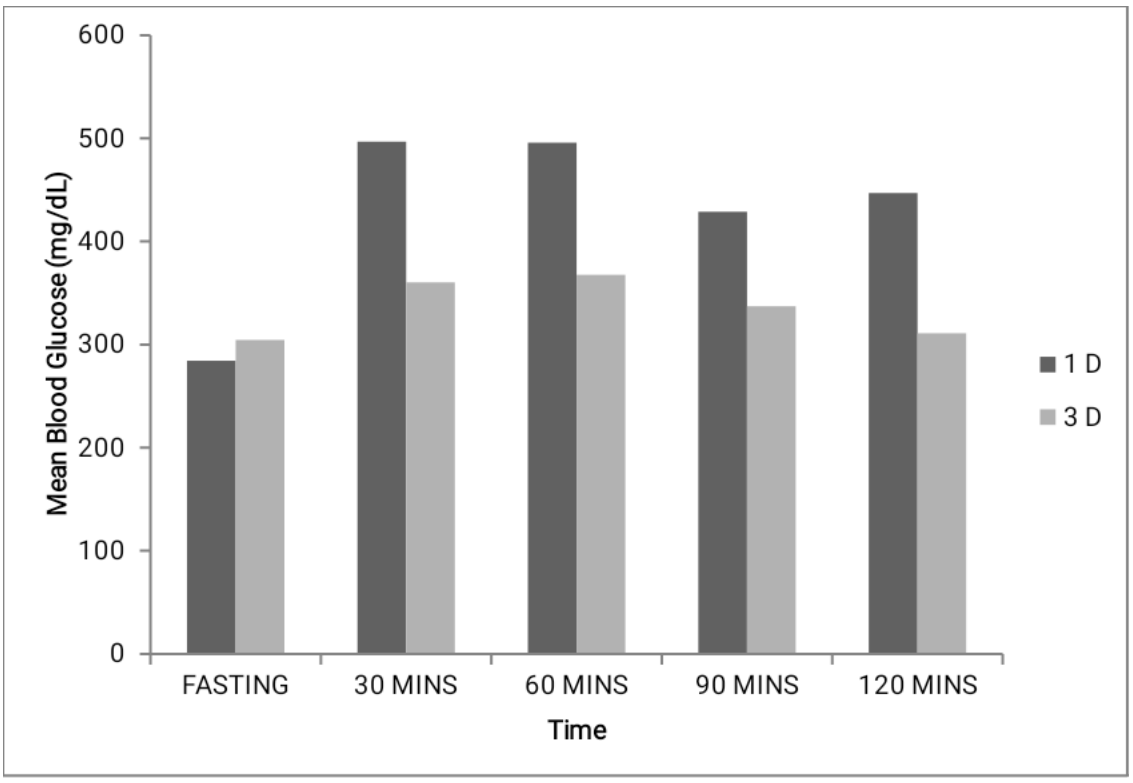
Figure 4.13 Oral Glucose Tolerance Test Result for Groups 1C and 3C

Group 1C= Diabetic rats with normal rat feed, Group 3C= Diabetic rats fed with 30% Cassava feed. Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for oral glucose tolerance tests in groups 1D and 3D are presented in figure 4.14 in the next page. From the figure, the following were observed;

The mean fasting blood glucose level of group 1D was significantly higher than group 3D which is an indication that concurrent intake of 30% cassava and iodine caused lowered fasting blood glucose level in diabetic rats in comparison with intake of normal rat feed and iodine.

The mean blood glucose levels in group 1D was significantly higher than group 3D after 30 minutes, 60 minutes and 90 minutes of glucose ingestion. There was no significant difference in mean blood glucose levels of group 1D and 3D after 120 minutes of glucose ingestion. This shows that concurrent intake of 30% cassava and iodine lowered blood glucose levels in the first 90 minutes of oral glucose tolerance test in diabetic rats in comparison to intake of normal rat feed and iodine.



p ≤ 0.05

Figure 4.14 Oral Glucose Tolerance Test Result for Groups 1D and 3D

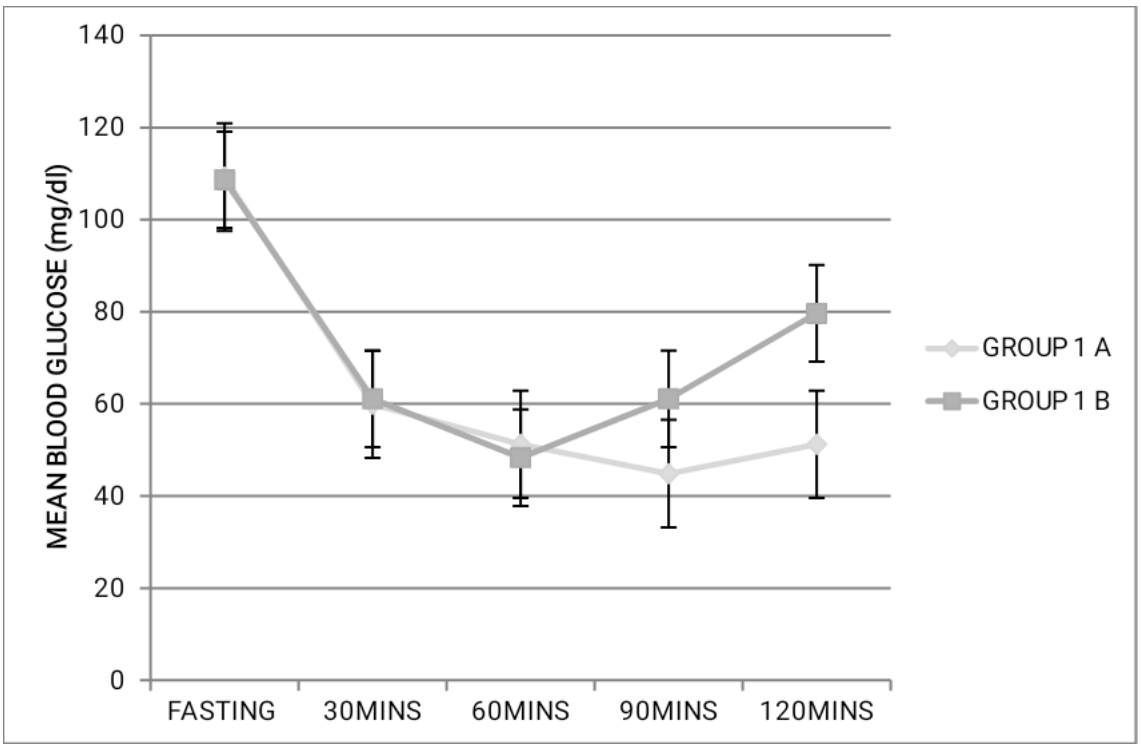
Group 1D= Diabetic rats with normal rat feed + Iodine (10mg/kg of feed), Group 3D= Diabetic rats fed with 30% Cassava feed + Iodine (10mg/kg of feed). Data are expressed in mean ±SEM (n=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

4.3 Results of Insulin Tolerance Tests

The results for insulin tolerance tests in groups 1A and 1B are presented in figure 4.15 in the next page. From the figure, the following were observed

There was no significant difference between the mean fasting blood glucose levels of groups 1A and 1B which implies that consumption of iodine had no significant effect on fasting blood glucose level of non-diabetic rats that consumed normal rat feed.

There was no significant difference between the mean fasting blood glucose levels of groups 1A and 1B after 30 minutes and 60 minutes of insulin injection. The mean blood glucose level of group 1A was significantly lower than group 1B 90 minutes and 120 minutes after insulin injection. This is an indication that intake of iodine did not alter insulin sensitivity in non-diabetic rats that consumed normal rat feed in the first 60 minutes of insulin tolerance test but blood glucose levels failed to fall after 90 minutes of insulin injection.



p ≤ 0.05

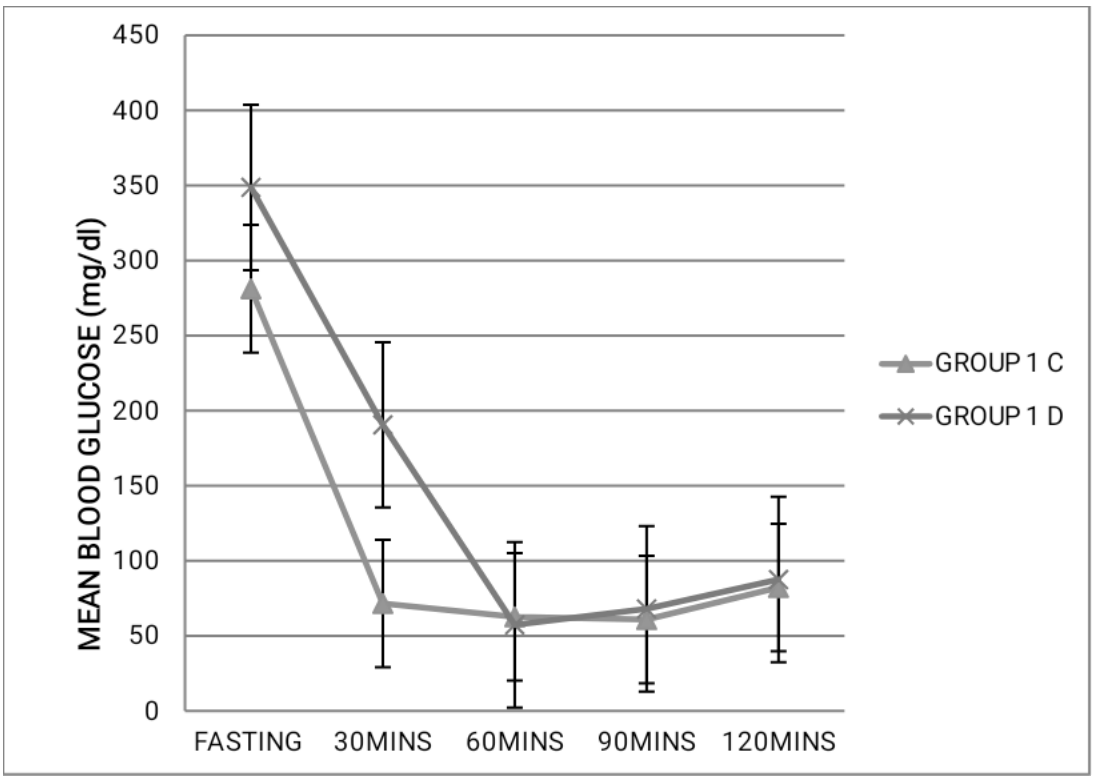
Figure 4.15 Insulin Tolerance Tests for Groups 1A and 1B

Group 1A= Non-diabetic rats with normal rat feed, Group 1B= Non-diabetic rats fed with normal rat feed + Iodine (10mg/kg of feed). Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for insulin tolerance tests in group 1C and 1D are presented in figure 4.16 in the next page. From the figure, the following were observed

There was no significant difference between the fasting blood glucose level of groups 1C and 1D which is an indication that iodine intake had no significant effect on mean fasting blood glucose level in diabetic rats that consumed normal rat feed.

The mean blood glucose level of group 1C was significantly lower than group 1D 30 minutes after insulin injection. There was no significant difference between the blood glucose level in group 1C and 1D after 60 minutes, 90 minutes and 120 minutes of insulin injection. This is an indication that intake of iodine and normal rat feed elevated mean blood glucose level 30 minutes in diabetic rats after insulin injection but had no significant effect on mean blood glucose levels after 60 minutes of insulin injection.



p ≤ 0.05

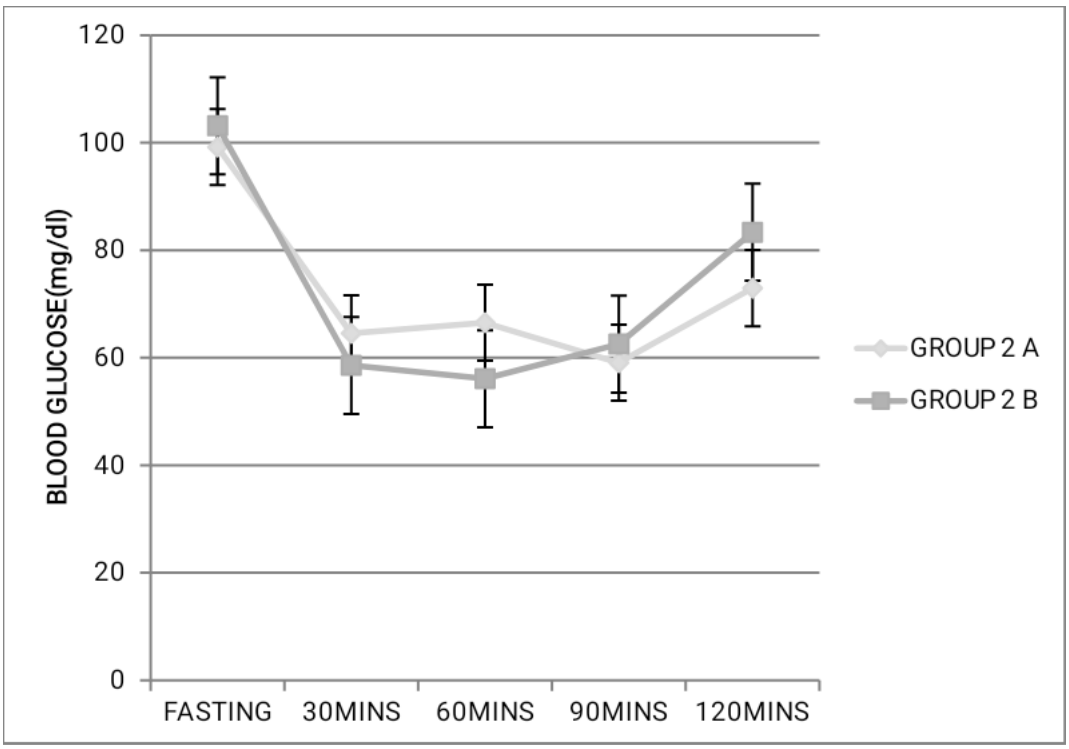
Figure 4.16 Insulin Tolerance Test for Groups 1C AND 1D

Group 1C=Diabetic rats with normal rat feed , Group 1D= Diabetic rats fed with normal rat feed + Iodine (10mg/kg of feed). Data are expressed in mean ±SEM (n=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for insulin tolerance tests in groups 2A and 2B are presented in figure 4.17 in the next page. From the figure, the following were observed;

There was no significant difference between the mean fasting blood glucose level of group 2A and 2B which is an indication that concurrent iodine and 30% soybeans intake had no significant effect on fasting blood glucose level in non-diabetic rats in comparison to intake of 30% soybeans without iodine.

There was no significant difference between the mean blood glucose level in group 2A and group 2B 30 minutes, 60 minutes, 90 minutes and 120 minutes after insulin injection. This is an indication that concurrent iodine and 30% soybeans intake did not significantly affect insulin sensitivity in non-diabetic rats in comparison to intake of 30% soybeans without addition of iodine.



p ≤ 0.05

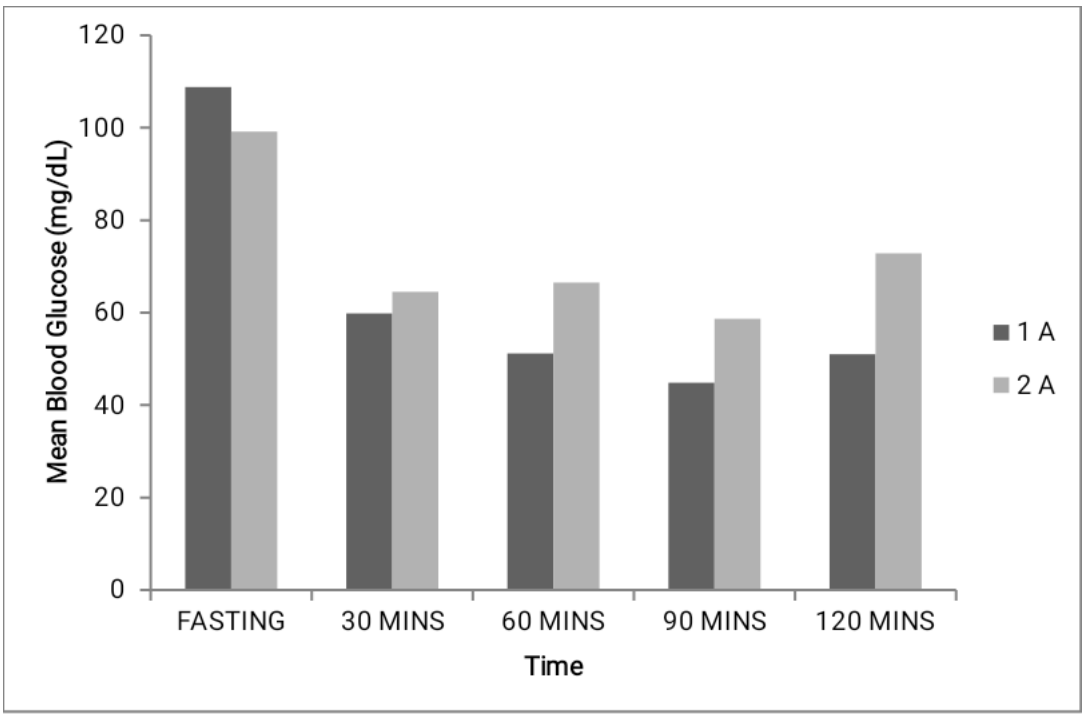
Figure 4.17 Insulin Tolerance Test Results for Groups 2A and 2B

Group 2A= Non-diabetic rats fed with 30% soybeans feed, Group 2B= Non-diabetic rats fed 30% soybeans feed + Iodine (10mg/kg of feed). Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for insulin tolerance tests for groups 1A and 2A are presented in figure 4.18 in the next page. From the figure, the following were observed;

The mean fasting blood glucose level of group 1A is significantly higher than group 2A which is an indication that 30% soybeans intake significantly lowered fasting blood glucose level in non-diabetic rats in comparison to intake of normal rat feed.

There was no significant difference in the mean blood glucose levels in group 1A and group 2A after 30 minutes and 60 minutes of insulin injection. The mean blood glucose level in group 1A was significantly lower than group 2A after 90 minutes and 120 minutes. This is an indication that although 30% soybeans intake did not significantly alter insulin sensitivity in non-diabetic rats for the first 60 minutes, it caused significantly elevated blood glucose levels after 90 minutes of insulin injection in comparison to intake of normal rat feed.



p ≤ 0.05

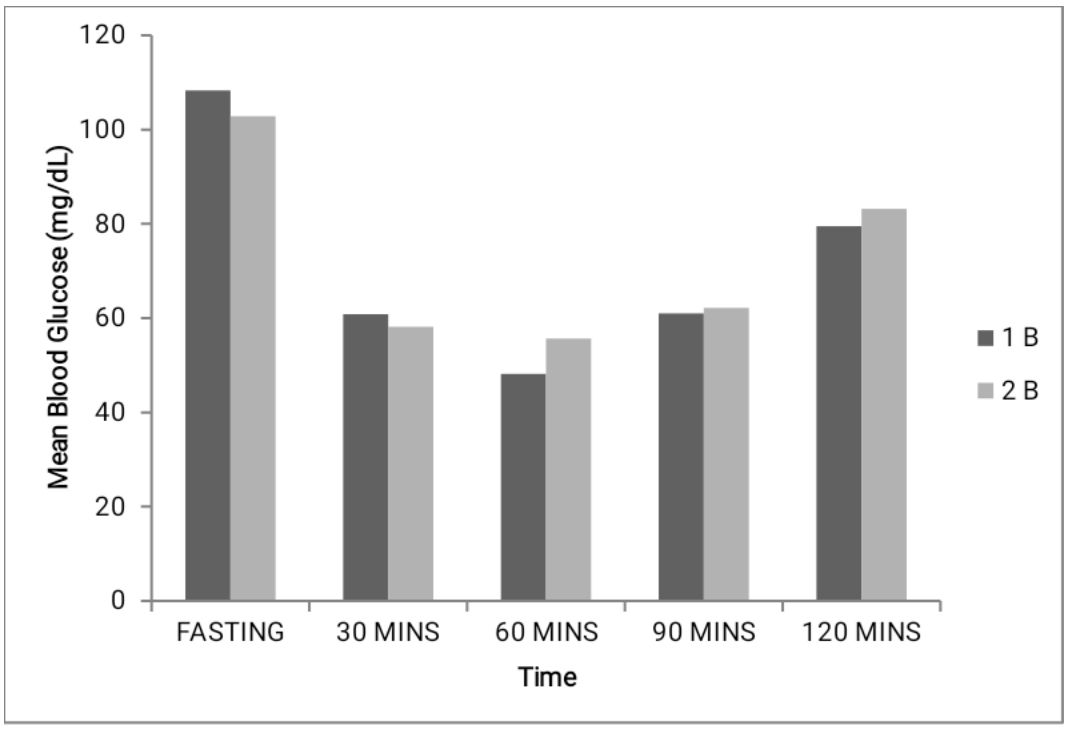
Figure 4.18 Insulin Tolerance Test Results for Groups 1A and 2A

Group 1A=Non-diabetic rats fed with normal rat feed ; Group 2A= Non-diabetic rats fed 30% soybeans feed. Data are expressed in mean ±SEM (n=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for insulin tolerance tests in groups 1B and 2B are presented in figure 4.19 in the next page. From the figure, the following were observed;

The mean fasting blood glucose level in group1B was significantly higher than in group 2B. This is an indication that concurrent iodine and 30% soybeans consumption did not significantly affect mean fasting blood glucose levels in non -diabetic rats in comparison with intake of iodine and normal rat feed.

There was no significant difference between the mean blood glucose levels in group 1B and 2B after 30 minutes, 60 minutes, 90 minutes and 120 minutes of insulin injection. This shows that concurrent intake of iodine and 30% soybeans did not alter insulin sensitivity in non-diabetic rats in comparison to iodine and normal rat feed intake.



p ≤ 0.05

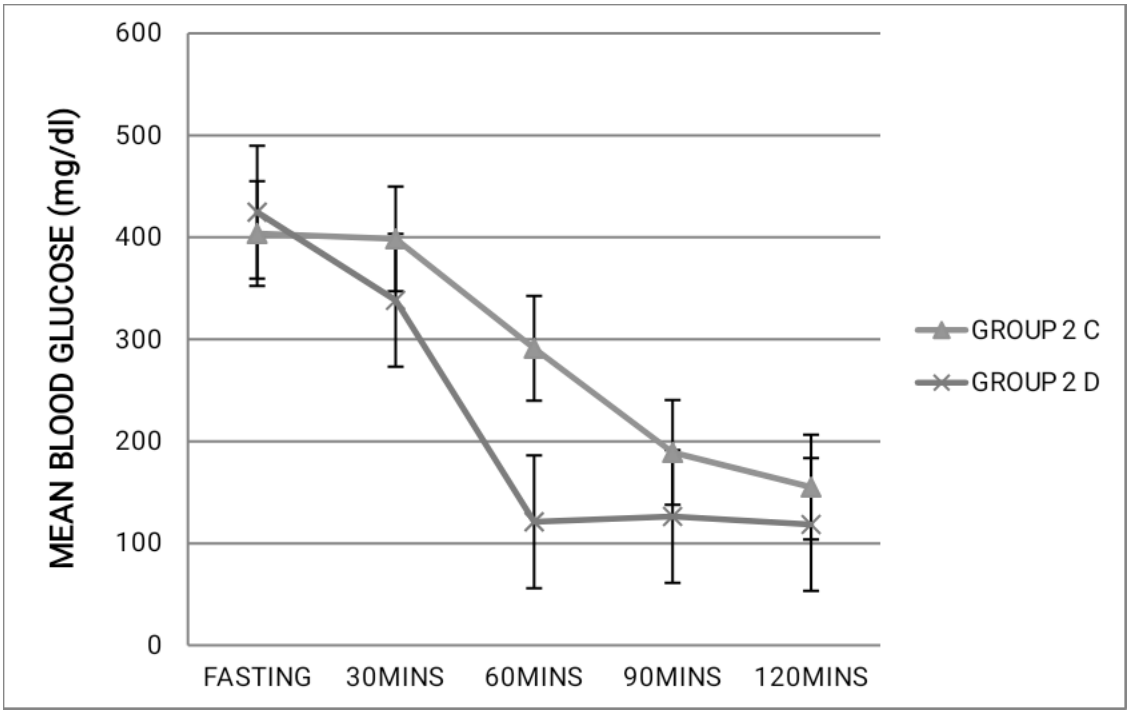
Figure 4.19 Insulin Tolerance Test Results for Groups 1B and 2B

Group 1B=Non-diabetic rats fed with normal rat feed + Iodine (10 mg/kg of feed); Group 2B= Non-diabetic rats fed 30% soybeans feed + Iodine (10 mg/kg of feed). Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for insulin tolerance tests in groups 2C and 2D are presented in figure 4.20 in the next page. From the figure, the following were observed;

There was no significant difference in the mean fasting blood glucose level of group 2C and 2D. This shows that concurrent iodine and 30% soybeans intake did not significantly affect mean fasting blood glucose level in diabetic rats when compared to intake of 30% soybeans without iodine addition.

The blood glucose level of group 2C was significantly higher than that of group 2D after 30 minutes, 60 minutes, 90 minutes and 120 minutes of insulin injection. This is an indication that iodine intake increased insulin sensitivity in diabetic rats that consumed 30% soybeans in comparison with the rats that were not fed with iodine.



p ≤ 0.05

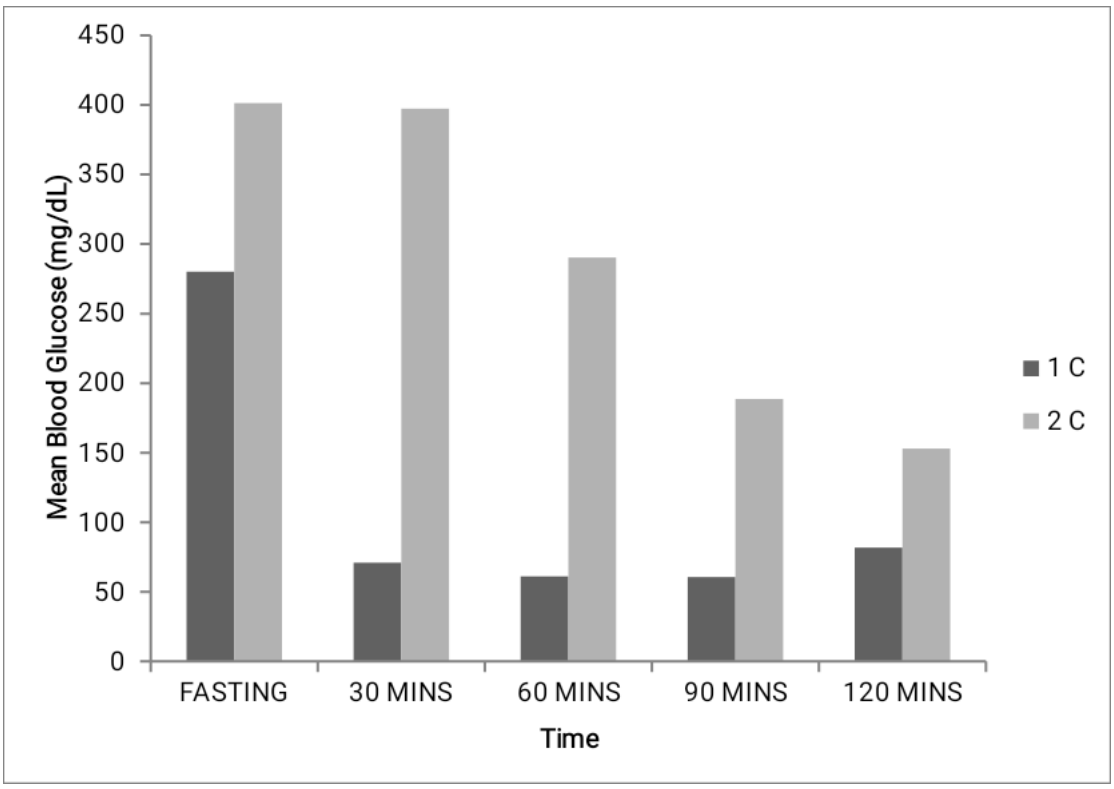
Figure 4.20 Insulin Tolerance Test Results for Groups 2C and 2D

Group 2C= Diabetic rats fed with 30% soybeans feed; Group 2D= Diabetic rats fed 30% soybeans feed + Iodine (10 mg/kg of feed). Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for the insulin tolerance tests in groups 1C and 2C are presented in figure 4.21 in the next page. From the figure, the following were observed;

The mean fasting blood glucose level of group 1C was significantly lower when compared to group 2C. This shows that ingestion of 30% soybeans significantly elevated mean fasting blood glucose level of diabetic rats in comparison to normal rat feed intake.

The mean blood glucose level in group 1C was significantly lower than group 2C throughout the insulin tolerance test. This is a pointer that intake of 30% soybeans reduced insulin sensitivity in diabetic rats when compared with intake of normal rat feed.



p ≤ 0.05

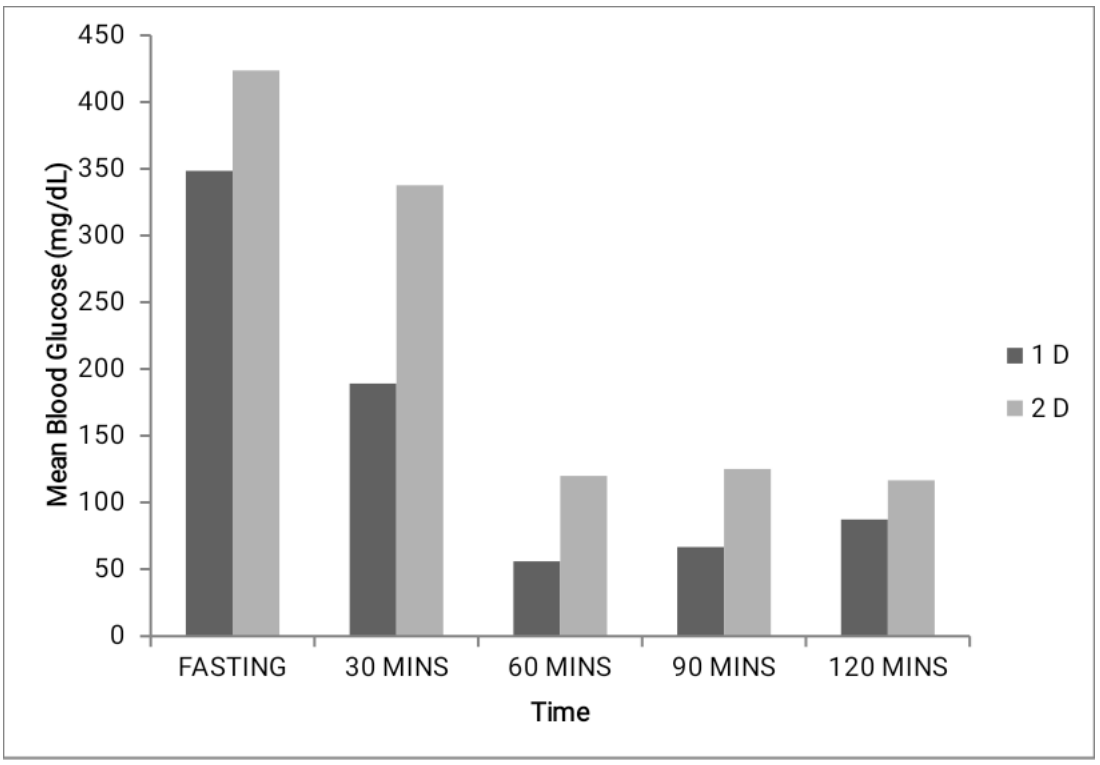
Figure 4.21 Insulin Tolerance Test Results for Groups 1C and 2C

Group 1C= Diabetic rats fed with normal rat feed; Group 2C= Diabetic rats fed 30% soybeans feed. Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for insulin tolerance tests in groups 1D and 2D are presented in figure 4.22 in the next page. From the figure, the following were observed;

The mean fasting blood glucose level of group 1D was significantly lower in comparison to group 2D. This indicates that concurrent intake of iodine and 30% soybeans significantly increased fasting blood glucose level in diabetic rats in comparison to intake of iodine and normal rat feed.

The mean blood glucose levels in group 1D were significantly lower than group 2D throughout the insulin tolerance test. This shows that concurrent consumption of iodine and 30% soybeans reduced insulin sensitivity when compared to intake of iodine and normal rat feed.



p ≤ 0.05

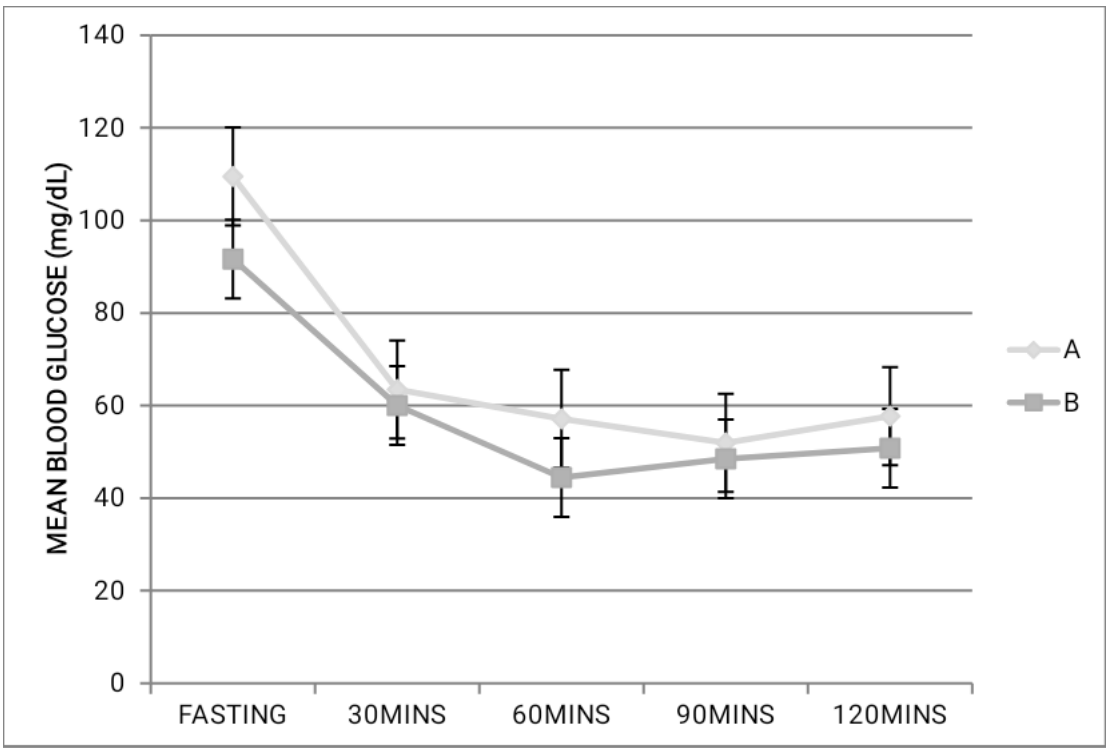
Figure 4.22 Insulin Tolerance Test Results for Groups 1D and 2D

Group 1D= Diabetic rats fed with normal rat feed + Iodine (10 mg/mg of feed); Group 2D= Diabetic rats fed with 30% soybeans + Iodine (10 mg/mg of feed). Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for insulin tolerance tests in groups 3A and 3B are presented in figure 4.23 in the next page. From the figure, the following were observed;

The mean fasting blood glucose level of group 3A was significantly higher than group 3B. This implies that concurrent consumption of iodine and 30% cassava significantly lowered fasting blood glucose level in comparison to consumption of 30% cassava without iodine treatment.

There was no significant difference between the mean blood glucose levels of group 3A and 3B 30 minutes after insulin injection. The mean blood glucose level of group 3A was significantly higher compared to group 3B 60 minutes after insulin infusion. There was no significant difference between the mean blood glucose levels of group 3A and 3B after 90 minutes and 120 minutes of insulin injection. This is an indication that concurrent iodine and 30% cassava intake significantly lowered blood glucose level 60 minutes after insulin injection but showed no significant effect on insulin sensitivity afterwards.



p ≤ 0.05

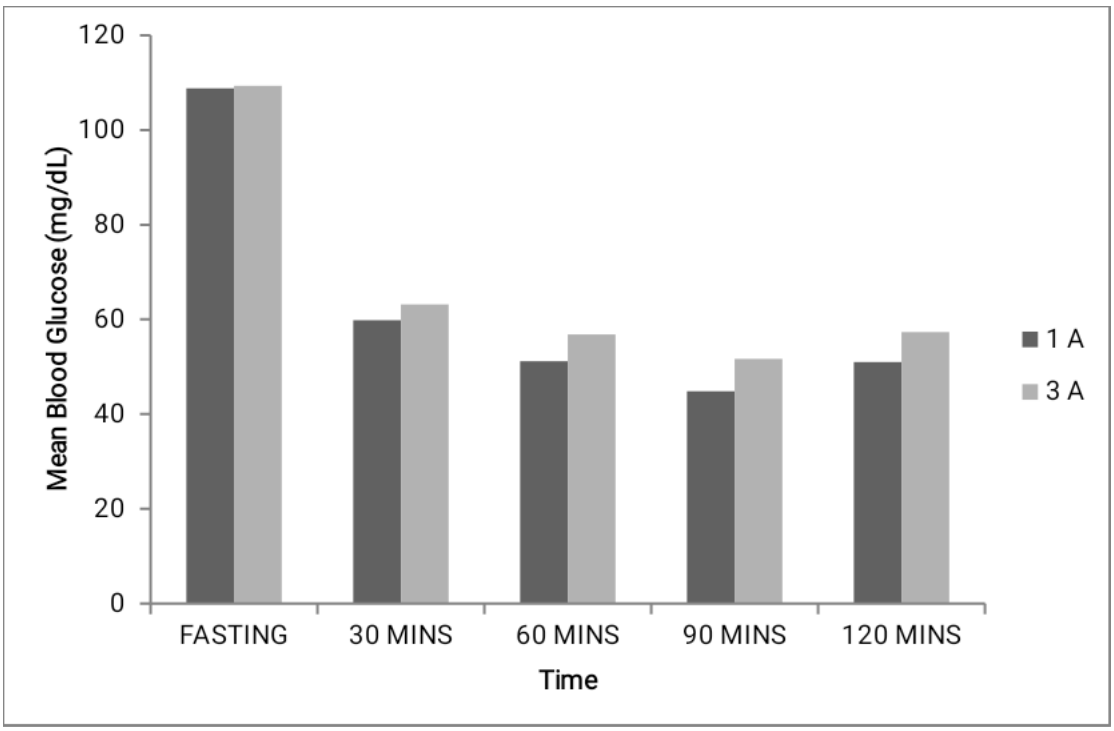
Figure 4.23 Insulin Tolerance Test Results for Groups 3A and 3B

Group 3A= Non-diabetic rats fed with 30% cassava feed; Group 3B= Non-diabetic rats fed 30% cassava feed + Iodine (10 mg/kg of feed). Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for insulin tolerance tests in groups 1A and 3A are presented in figure 4.24 in the next page. From the figure, the following were observed;

There was no significant difference between the mean fasting blood glucose level of group 1A and 3A. This implies that consumption of 30% cassava did not significantly affect mean fasting blood glucose level in non-diabetic rats when compared to intake of normal rat feed.

There was no significant difference in the mean blood glucose levels of group1A and 3A 30 minutes, 60 minutes and 120 minutes after insulin injection. The mean blood glucose level in group 1A was significantly lower than group 3A 90 minutes after insulin injection. This is an indication that intake of 30% cassava had no significant effect on insulin sensitivity 30 minutes and 60 minutes after insulin injection in non-diabetic rats but showed a tendency to recover more quickly from the hypoglycaemic effect of insulin (at 90 minutes) when compared to intake of normal rat feed.



p ≤ 0.05

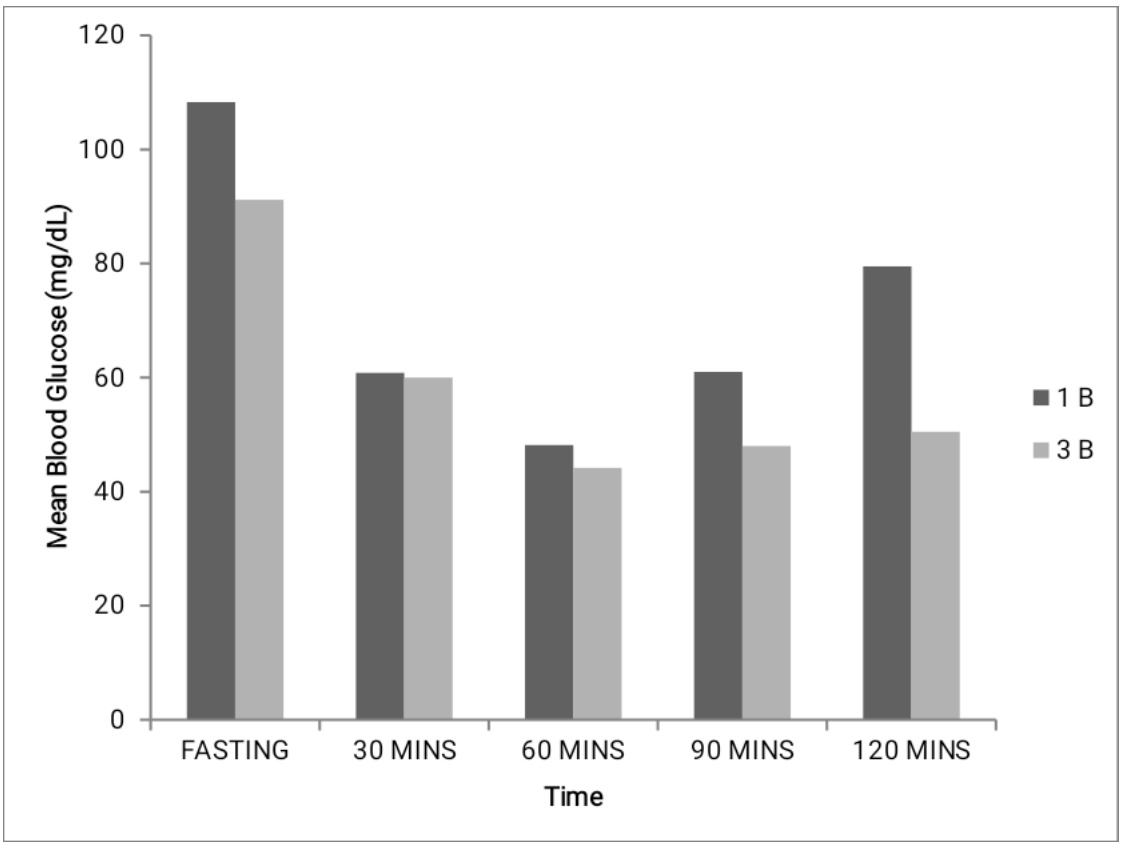
Figure 4.24 Insulin Tolerance Test Results for Groups 1A and 3A

Group 1A= Non-diabetic rats fed with normal rat feed; Group 3A= Non-diabetic rats fed 30% cassava feed. Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for insulin tolerance tests in groups 1B and 3B are presented in figure 4.25 in the next page. From the figure, the following were observed;

The mean fasting blood glucose of group 1B was significantly higher than group 3B. This implies that concurrent iodine and 30% cassava intake significantly lowered mean fasting blood glucose level in non-diabetic rats when compared with consumption of iodine and normal rat feed.

There was no significant difference between the mean blood glucose levels of group 1B and 3B after 30 minutes, 60 minutes and 90 minutes of insulin administration. The mean blood glucose level of group 1B was significantly higher than group 3B after 120 minutes. This is an indication that hypoglycaemic action of insulin lasted longer in non-diabetic rats that were fed with iodine and 30% cassava in comparison to those fed with iodine and normal rat feed.



p ≤ *0.05*

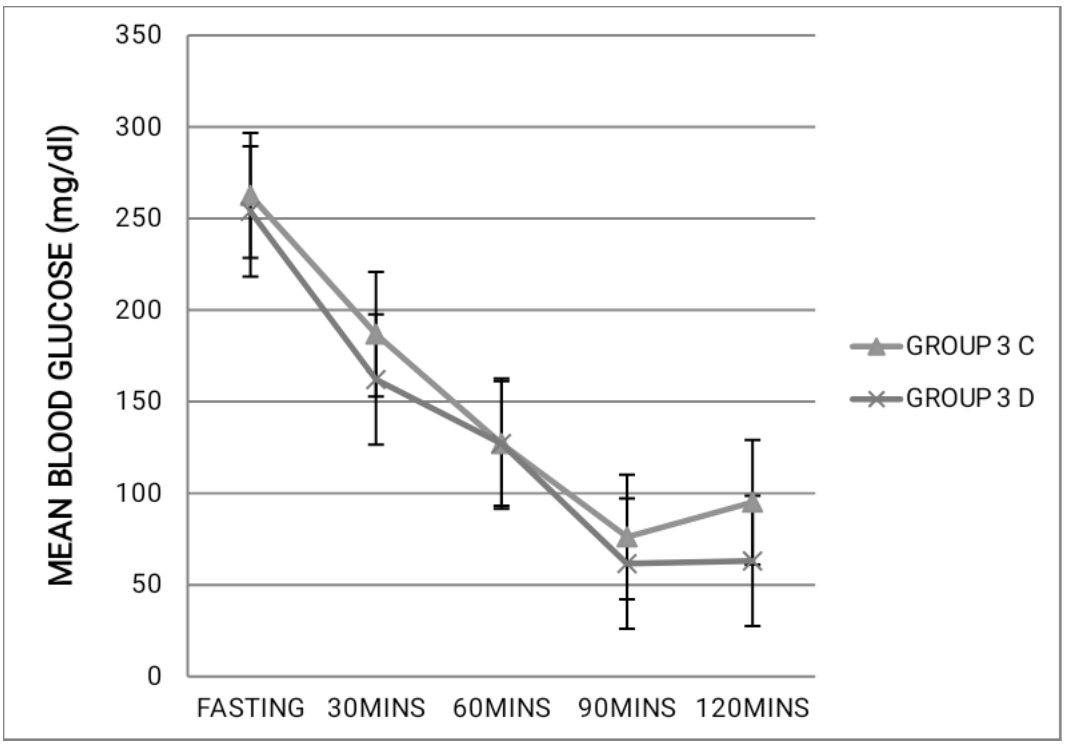
Figure 4.25 Insulin Tolerance Test Results for Groups 1B and 3B

Group 1B= Non-diabetic rats fed with normal rat feed + Iodine (10 mg/kg of feed); Group 3B= Non-diabetic rats fed 30% cassava feed + Iodine (10 mg/kg of feed). Data are and students 'T test' for unpaired groups.

The results for insulin tolerance tests in groups 3C and 3D are presented in figure 4.26 in the next page. From the diagram, the following were observed;

There was no significant difference between the mean fasting blood glucose level of group 3C and 3D. This implies that concurrent iodine and 30% cassava intake had no significant effect on the mean fasting blood glucose level in diabetic rats when compared to intake of 30% cassava without iodine treatment.

The mean blood glucose level of group 3C was significantly higher than group 3D after 30 minutes, 90 minutes and 120 minutes of insulin injection. There was no significant difference between mean blood glucose levels in group 3C and 3D after 60 minutes. This is an indication that concurrent intake of iodine and 30% cassava significantly increased insulin sensitivity in diabetic rats.



p ≤ 0.05

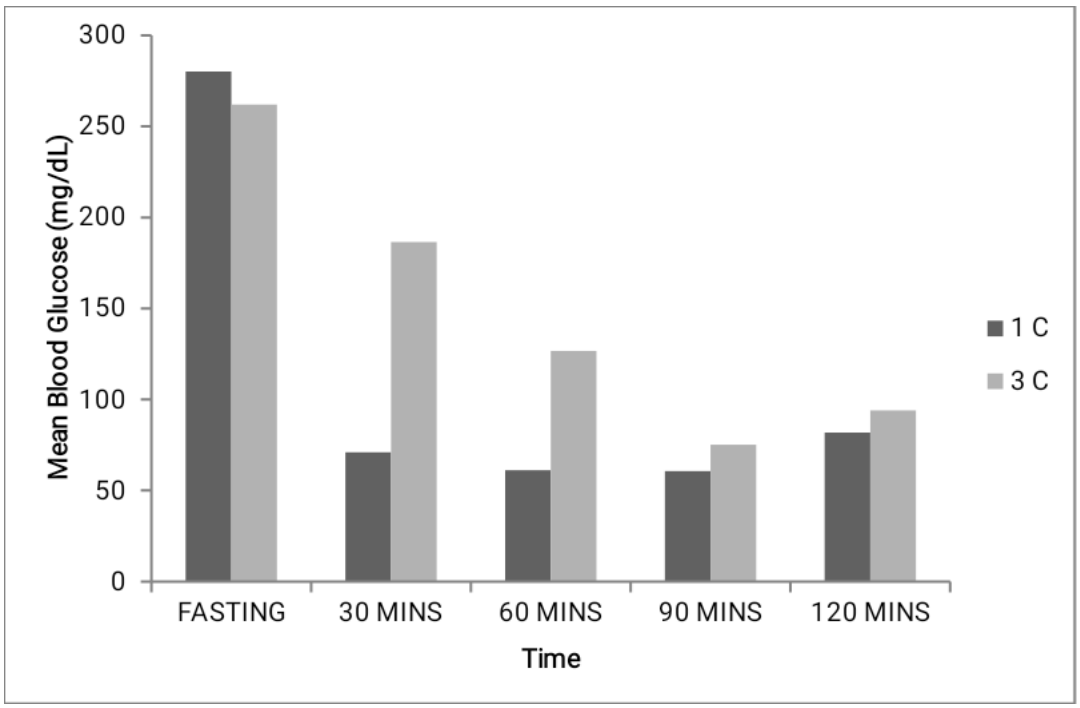
Figure 4.26 Insulin Tolerance Test Results for Groups 3C and 3D

Group 3C= Diabetic rats fed with 30% cassava feed; Group 3D= Diabetic rats fed 30% cassava feed + Iodine (10 mg/kg of feed). Data are expressed in mean ±SEM (n=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for insulin tolerance tests in groups 1C and 3C are presented in figure 4.27 in the next page. From the figure, the following were observed;

There was no significant difference between the mean fasting blood glucose level in group 1C and 3C. This implies that consumption of 30% cassava did not significantly alter mean fasting blood glucose level in diabetic rats when compared with intake of normal rat feed.

The mean blood glucose levels in group 1C were significantly lower than in group 3C after 30 minutes, 60 minutes and 90 minutes after insulin administration. There was no significant difference in the mean blood glucose levels of group 1C and group 3C after 120 minutes of insulin administration. This is an indication that consumption of 30% cassava significantly decreased insulin sensitivity in diabetic rats in comparison to consumption of normal rat feed.



p ≤ 0.05

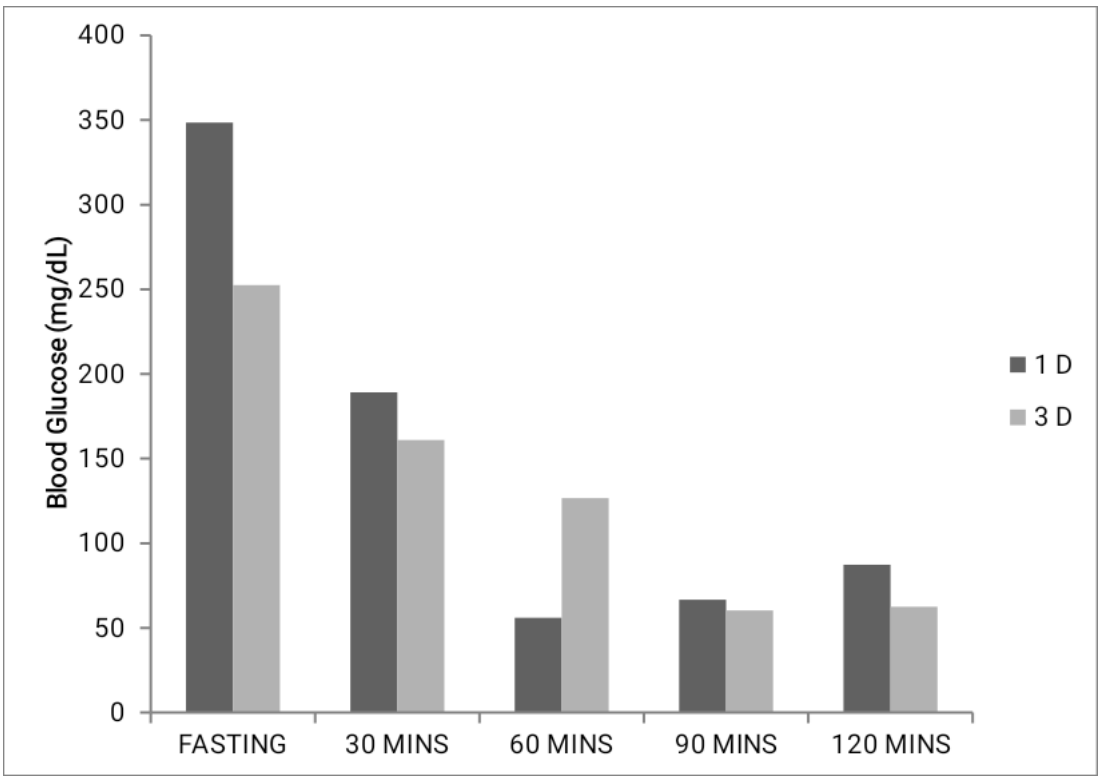
Figure 4.27 Insulin Tolerance Test Results for Groups 1C and 3C

Group 1C= Diabetic rats fed with normal rat feed; Group 3C= Diabetic rats fed 30% cassava feed. Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results for insulin tolerance tests in groups 1D and 3D are presented in figure 4.28 in the next page. From the figure, the following were observed;

The mean fasting blood glucose level in group 1D was significantly higher than group 3D. This implies that concurrent consumption of iodine and 30% cassava significantly lowered mean fasting blood glucose levels in diabetic rats in comparison with iodine and normal rat feed intake.

The mean blood glucose level in group 1D was significantly higher than group 3D throughout the insulin tolerance test. This is an indication that in diabetic rats, concurrent iodine and cassava intake significantly increased insulin sensitivity in comparison to iodine and normal rat feed intake.



p ≤ 0.05

Fig. 4.28 Insulin Tolerance Test Results for Groups 1D and 3D

Group 1D=Diabetic rats fed with normal rat feed + iodine; Group 3D= Diabetic rats fed 30% cassava feed + iodine. Data are expressed in mean ±SEM (n=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

4.4 Results of Insulin Resistance

The results for insulin resistance are presented in table 4.3 in the next page. From the table, the following was observed;

From the calculations for insulin resistance (HOMA-IR equation), insulin resistance was observed in group 3C. This shows that the type 1 diabetic rats fed with 30% cassava developed insulin resistance.

Table 4.3 Results of Insulin Resistance (HOMA-IR)

Groups	A	B	C	D
1	0.4	0.1	0.2	0.2
2	1.2	1.3	0.2	0.3
3	0.2	0.6	4.8*	0.3

Group 1= Normal rat feed; Group 2= 30% soybeans feed, Group 3= 30% cassava feed. (Subgroups; A= Non-diabetic, B= Non-diabetic + Iodine (10mg/kg of feed), C= Diabetic, D= Diabetic + Iodine (10mg/kg of feed)). Data are expressed in mean ±SEM (*n*=5). The symbol * denotes Insulin resistance

The mean serum insulin levels are presented in table 4.4. From the table, the following were observed

The mean serum insulin level of group 1A was significantly higher than group 1B. There was no significant difference in the mean serum insulin level of group 1C and group 1D. This is an indication that iodine intake significantly lowered mean serum insulin levels in non-diabetic rats fed with normal rat feed but had no did not significantly alter mean serum insulin concentration in diabetic rats.

There was no significant difference between the mean serum insulin level of group 2A and group 2B. The mean serum insulin level in group 2C was significantly lower than in group 2D. This implies that iodine intake did not significantly affect mean serum insulin level in non-diabetic rats that were fed with 30% soybeans but significantly increased it in diabetic rats.

The mean serum insulin level in group 3A was significantly lower than group 3B. The mean serum insulin level in group 3C was significantly higher than group 3D. This implies that iodine consumption caused elevated mean serum insulin concentration in non-diabetic rats that were fed with 30% cassava but lowered serum insulin levels in diabetic rats.

The mean insulin level in group 1A was significantly lower than group 2A and higher than 3A. This shows that consumption of 30% soybeans significantly increased mean serum insulin concentrations and intake 30% cassava significantly lowered serum insulin level in non-diabetic rats in comparison with consumption of normal rat feed.

The mean insulin level in group 1B was significantly lower than groups 2B and 3B. This is an indication that concurrent intake of iodine and 30% soybeans or 30% cassava caused significantly higher mean serum insulin concentrations in non-diabetic experimental rats in comparison to intake of iodine and normal rat feed.

The mean insulin level in group 1C was significantly higher than group 2C and significantly lower than group 3C. This implies that in diabetic rats, intake of 30%

soybeans significantly lowered serum insulin level and consumption of 30% cassava significantly increased mean serum insulin level in comparison with normal rat feed consumption.

The mean serum insulin level of group 1D was significantly lower than group 2D. There was no significant difference in the mean insulin levels of groups 1D and 3D. This is an indication that diabetic rats that consumed iodine and 30% soybeans had significantly higher mean serum insulin concentration and those fed with 30% cassava and iodine showed no significant alterations in their mean serum insulin concentration in comparison to diabetic rats that consumed normal rat feed and iodine.

Table 4.4 Mean serum Insulin levels (Iμ/mL)

Groups	A	B	C	D
1	1.48±0.16^*	0.45±0.09	0.23±0.03^	0.18±0.03
2	5.25±0.15^	5.00±0.54^	0.17±0.03	0.30±0.04^*
3	0.92±0.03	2.63±0.17^*	5.65±0.35*^	0.43±0.12

Group 1= Normal rat feed; Group 2= 30% soybeans feed; Group 3= 30% cassava feed. (Subgroups; A= Non-diabetic, B= Non-diabetic + Iodine (10mg/kg of feed), C= Diabetic, D= Diabetic + Iodine (10mg/kg of feed)). Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students 'T test'

for unpaired groups. The symbol ^ denotes $p \leq 0.05$ significantly higher in groups 1and 2 or 1and 3. The symbol *denotes $p \leq 0.05$ significantly higher in groups A and B or C and D.

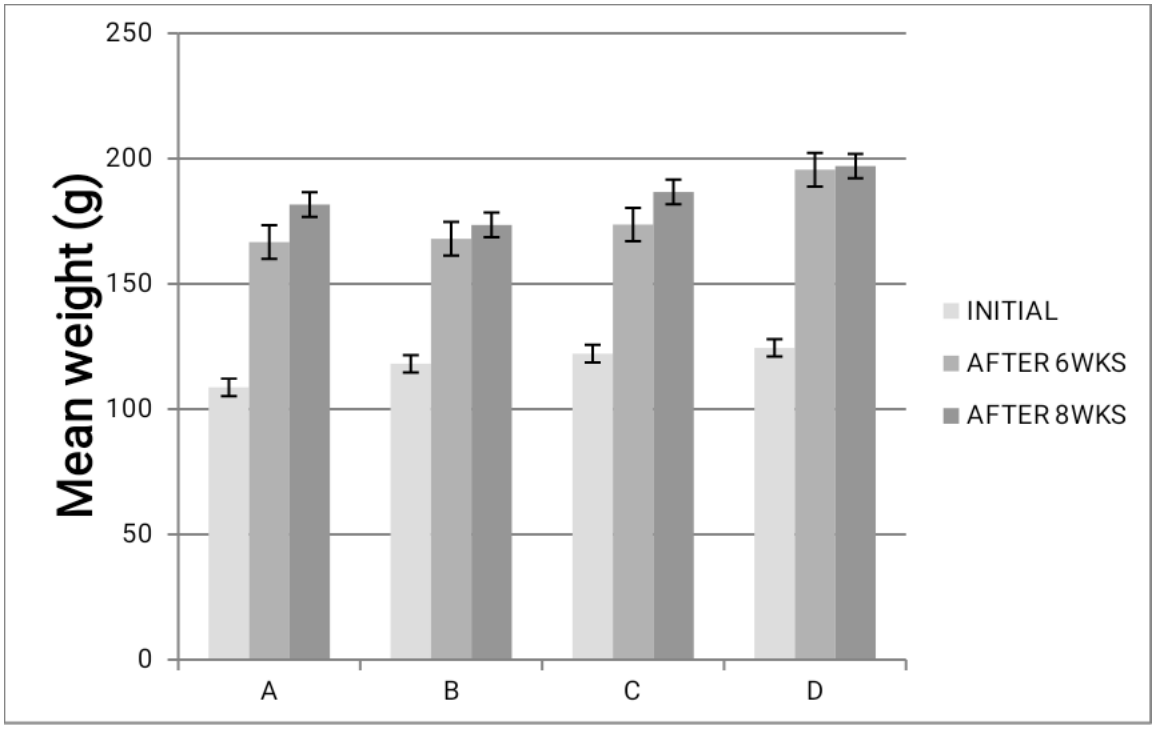
4.5 Results of Mean Body Weights

The results of the mean body weights (initial, after 6 weeks and after 8 weeks) in group 1 are presented in figure 4.29 in the next page. From the figure, the following were observed;

There was no significant difference in the weight gained in group 1A and 1B after 6 weeks and at the end (8 weeks) of the experiment. This is an indication that iodine intake had no significant effect on body weight in non-diabetic rats that consumed normal rat feed.

There was no significant difference in weight gained in group 1C and 1D after 6 weeks. Group 1D gained significantly higher weight than group 1C before the end of the experiment. `This is an indication that in diabetic rats fed with iodine and normal rat feed, there was significantly higher weight gain after induction of diabetes in comparison with those that were fed with normal rat feed without iodine treatment.

`



p ≤0.05

Figure 4.29 Body Weights (Initial, after 6 Weeks and after 8 weeks) In Group

1(Normal rat feed).

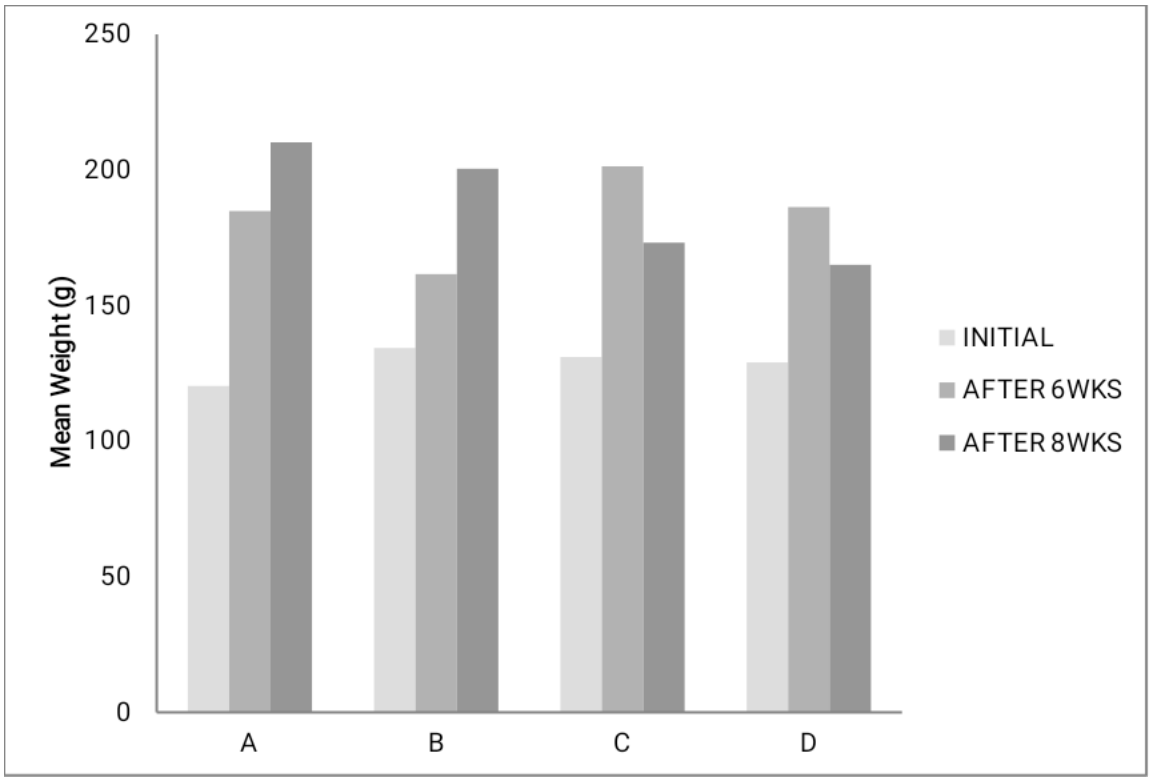
Subgroups; A= Non-diabetic, B= Non-diabetic + Iodine (10mg/kg of feed), C= Diabetic, D= Diabetic + Iodine (10mg/kg of feed). Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students' T test for unpaired groups.

The results for mean body weights in group 2 were presented in figure 4.30 in the next page. From the figure, the following were observed;

The mean body weight gain in group 2A after 6 weeks was significantly higher than that of group 2B. There was no significant difference between the weight gained in group 2A and 2B after 8 weeks. This is an indication that iodine intake caused significantly lower mean body weight gain in non-diabetic rats fed with 30% soybeans for the first 6 weeks of the experiment but showed no significant effect on mean body weight gain at the end of the experiment in comparison to intake of 30% soybeans intake without iodine treatment.

There was no significant difference between the weight change in group 2C and 2D after 6 weeks. There was no significant difference in the weight loss in group 2C and 2D after 8 weeks. This indicates that there was weight loss among the diabetic rats fed with 30% soybeans. Iodine intake did not significantly influence

the weight loss of the experimental rats.



p ≤0.05

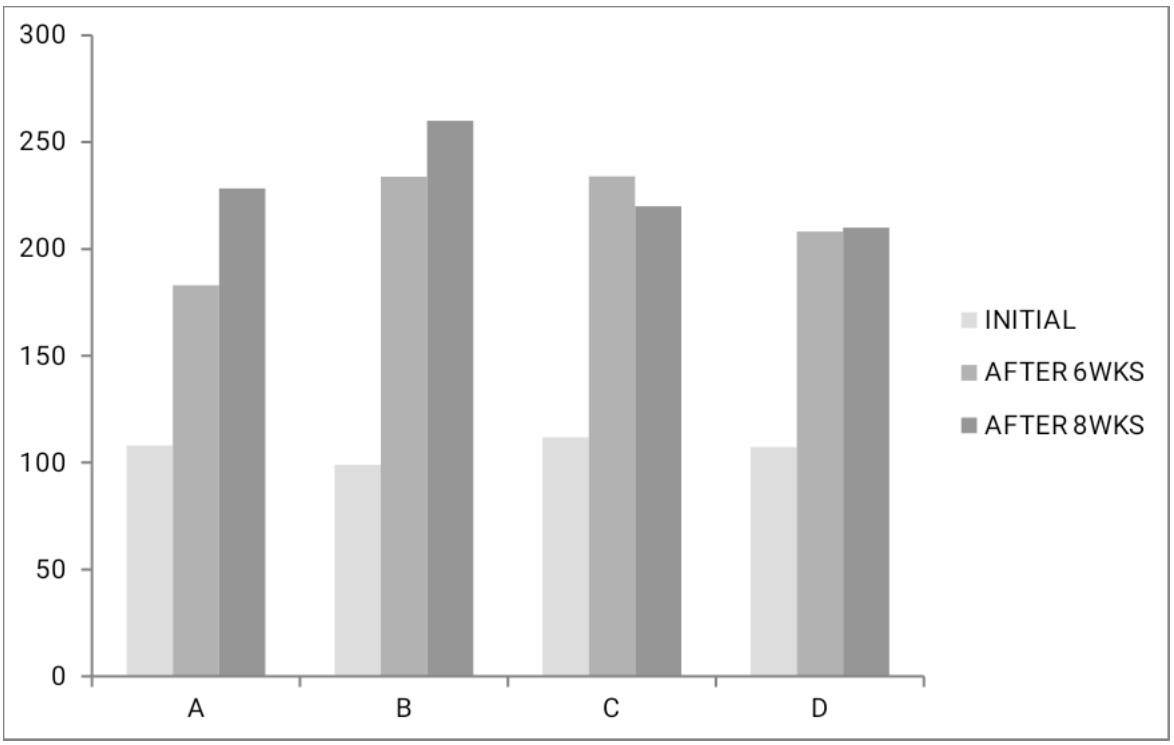
Figure 4.30 Mean Body Weights (Initial, after 6 Weeks and 8 weeks) In Group 2 (30% soybeans feed).

Subgroups; A= Non-diabetic, B= Non-diabetic + Iodine (10mg/kg of feed), C= Diabetic, D= Diabetic + Iodine (10mg/kg of feed). Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

The results of mean body weights in group 3 are presented in figure 4.31 in the next page. From the figure, the following were observed

The weight gain in group 3A was significantly lower than group 3B after 6 weeks and 8 weeks. This is an indication that concurrent intake of iodine and 30% cassava caused significantly higher mean body weight in non-diabetic rats when compared to intake of 30% cassava without iodine treatment.

There was no significant difference in the weight changes in group 3C and 3D after 6 weeks and 8 weeks. This shows that in diabetic rats that consumed both iodine and 30% cassava, there was no significant difference in the mean body weight when compared with diabetic rats that consumed 30% cassava without iodine treatment.



p ≤0.05

Figure 4.31 Mean Body Weights (Initial, after 6 Weeks and 8 weeks) In Group 3 (30% cassava feed).

Subgroups; A= Non-diabetic, B= Non-diabetic + Iodine (10mg/kg of feed), C= Diabetic, D= Diabetic + Iodine (10mg/kg of feed). Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students' T test for unpaired groups.

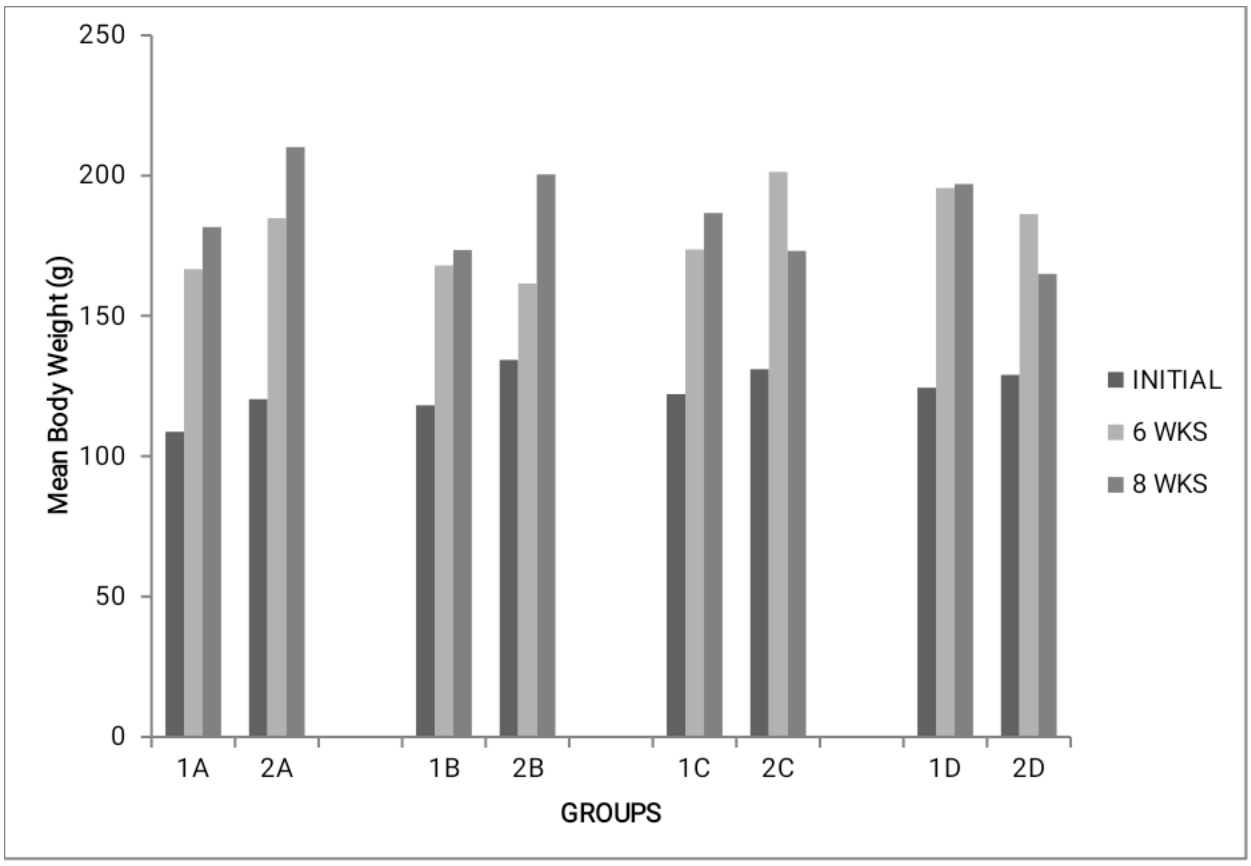
The results for mean body weights in groups 1A and 2A are presented in figure 4.32 in the next page. From the figure, the following were observed;

There was no significant difference between the weight changes in group1A and group 2A after 6 weeks and 8weeks. This indicates that 30% soybeans intake did not significantly alter body weight in non-diabetic rats.

The weight gain in group 1B was significantly higher than group 2B after 6 weeks. There was no significant difference between the mean weight gain in group 1B and 2B after 8weeks. This is an indication that concurrent iodine and 30% soybeans intake significantly reduced mean body weight gain in non-diabetic rats in the first 6 weeks of the experiment when compared with intake of normal rat feed and iodine.

There was no significant difference in weight gain in group 1C and group 2C. There was a significantly higher weight gain in group 1C than in group 2C after 8weeks. This is an indication that intake of 30% soybeans significantly reduced weight gain in diabetic rats in comparison with intake of normal rat feed.

There was no significant difference between the weight gained in group 1D and group 2D after 6weeks. The weight gain in group 1D was significantly higher than in group 2D after 8weeks. This is an indication that in diabetic rats, concurrent iodine and 30% soybeans intake significantly decreased weight gain in comparison to intake of normal rat feed and iodine.



p ≤0.05

Figure 4.32 Mean body weights of rats in groups 1(Normal rat feed) and 2 (30% soybeans feed).

Subgroups; A= Non-diabetic, B= Non-diabetic + Iodine (10mg/kg of feed), C= Diabetic, D= Diabetic + Iodine (10mg/kg of feed). Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

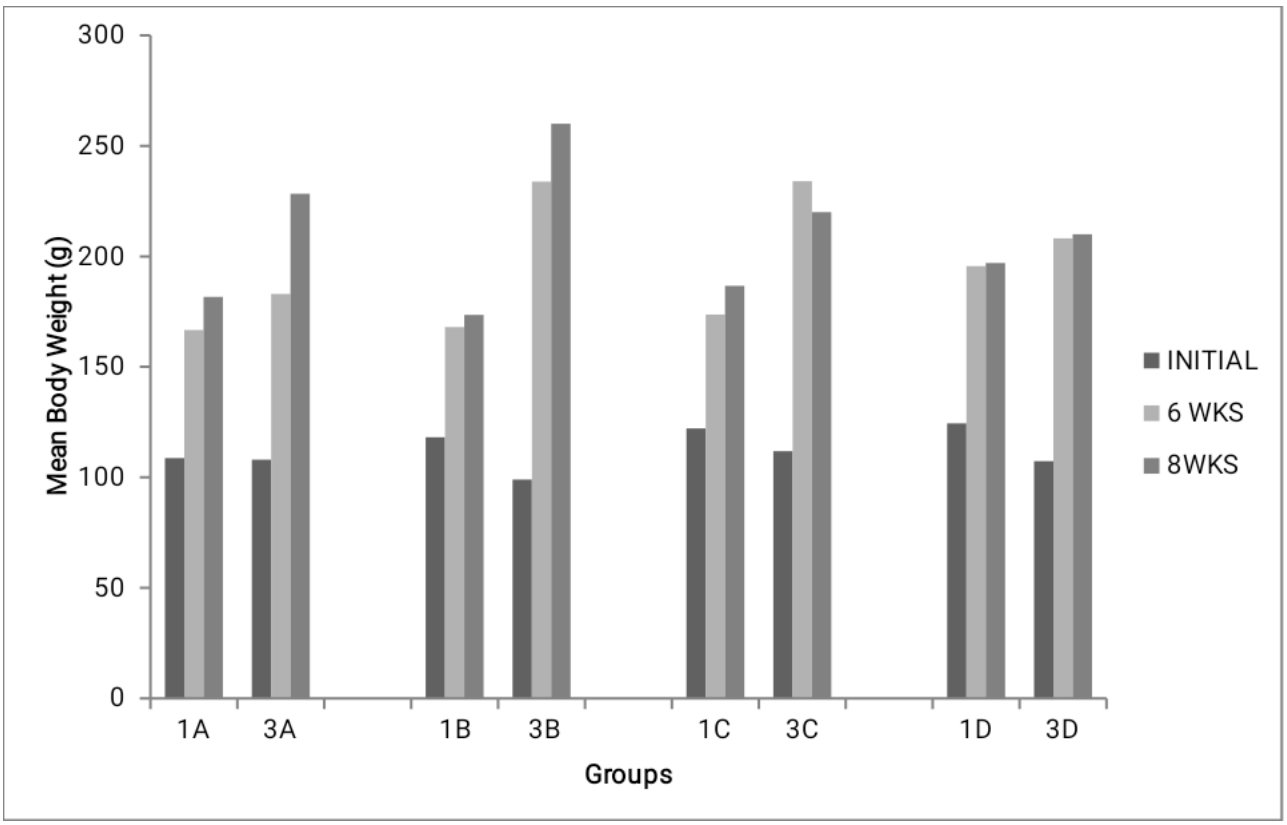
The results for mean body weights in groups 1A and 3A are presented in figure 4.33 in the next page. From the figure, the following were observed;

There was no significant difference between the mean body weight gains in groups 1A and 3A after 6 weeks. The mean weight gain in group 1A was significantly lower than 3A after 8 weeks of the experiment. This is an indication that 30% cassava intake significantly increased mean body weight in non-diabetic rats.

The weight gain in group 1B was significantly lower than group 3B 6weeeks after. The weight difference in group 1B was significantly lower than group 3B after 8 weeks. This implies that concurrent intake of iodine and 30% cassava significantly increased body weight in non-diabetic rats in comparison to intake of normal rat feed and iodine.

There was a significantly lower weight gain in group 1C than group 3C after 6 weeks. There was significantly lower weight gain in group 1C than group 3C after 8weeks. This shows that intake of cassava caused significantly higher mean body weight in diabetic rats.

The weight gain in group 1D was significantly lower than group 3D after 6weeks. There was no significant difference between weight gain in group 1D and group 3D 8weeks after. This is an indication that concurrent intake of iodine and 30% cassava did not significantly affect mean body weight in diabetic rats after diabetes was induced on the 6^{th} week of the experiment in comparison to intake of normal rat feed and iodine.



p ≤0.05

Figure 4.33 Mean body weights of rats in groups 1 (Normal rat feed) and 2 (30% soybeans feed).

Subgroups; A= Non-diabetic, B:=Non-diabetic + Iodine (10mg/kg of feed), C= Diabetic, D= Diabetic + Iodine (10mg/kg of feed). Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups.

4.6 Results for Thyroid Function Tests

The results for mean T_3 (triidothyronine) levels are presented in table 4.5 in the next page. From the table, the following were observed;

There was no significant difference in the mean T_3 level of group 1A and group 1B. There was no significant difference in the mean T_3 level of group 1C and group 1D. This is an indication that iodine intake did not significantly affect mean serum T_3 level in non-diabetic rats and diabetic rats fed with normal rat feed.

The mean T_3 level of group 2A was significantly higher than group 2B. There was no significant difference in the mean T_3 level of group 2C and group 2D. This implies that concurrent intake of iodine and 30% soybeans caused significantly decreased mean serum T_3 level in non-diabetic rats but had no significant effect on mean serum T_3 level in diabetic rats in comparison to intake of 30% soybeans without iodine treatment.

The mean T_3 level of group 3A was significantly higher than group 3B. There was no significant difference in the mean T_3 level of group 3C and group 3D. This implies that concurrent intake of iodine and 30% cassava caused significantly decreased mean serum T_3 level in non-diabetic rats but had no significant effect on mean serum T_3 level in diabetic rats in comparison to intake of 30% cassava without iodine treatment.

The mean T_3 level of group 1A was significantly lower than group 2A. This is an indication that intake of 30% soybeans significantly increased mean serum T_3 level in non-diabetic rats in comparison with intake of normal rat feed.

There was no significant difference in the mean T_3 levels of group 1B and group 2B. This implies that concurrent intake of iodine and 30% soybeans had no significant effect on mean serum T_3 level in non-diabetic rats in contrast with normal rat feed and iodine intake.

There was no significant difference between the mean T_3 levels of group 1C and group 2C. There was no significant difference between the mean serum T_3 levels of group 1D and 2D. This is an indication that intake of 30% soybeans and concurrent iodine and 30% soybeans intake had no significant effect on the mean serum T_3 level in diabetic rats when compared with intake of normal rat feed.

There was no significant difference between the mean T_3 levels of group 1A and group 3A. This shows that intake of 30% cassava had no significant effect on mean serum T_3 level in non-diabetic rats in comparison to intake of normal rat feed.

There was no significant difference between the mean T_3 levels of group 1B and group 3B. This shows that concurrent intake of 30% cassava and iodine did not significantly affect mean serum T_3 level in non-diabetic rats when compared with intake of normal rat feed and iodine.

There was no significant difference in the mean T_3 levels of group 1C and group 3C. There was no significant difference in the mean serum T_3 levels of group 1D and 3D. This is an indication that intake of 30% cassava and concurrent intake of iodine and 30% cassava had no significant effect on mean serum T_3 level in diabetic rats in comparison to intake of normal rat feed.

Table 4.5 Mean SerumT_3 Levels (ng/mL)

Groups	A	B	C	D
1	2.98±0.26	2.38±0.0003	3.18±0.08	2.03±0.5
2	6.75±0.06^*	2.90±0.19	2.02±0.12	2.35±0.15
3	3.48±0.10*	2.86±0.04	2.48±0.55	2.77±0.18

Group 1= Normal rat feed; Group 2= 30% soybeans feed, Group 3= 30% cassava feed. (Subgroups; A= Non-diabetic, B= Non-diabetic + Iodine (10mg/kg of feed), C= Diabetic, D= Diabetic + Iodine (10mg/kg of feed)). Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups. The symbol ^ denotes $p \leq 0.05$ significantly higher in groups 1and 2 or 1and 3. The symbol *denotes $p \leq 0.05$ significantly higher in groups A and B or C and D.

The results for mean serum T_4 levels are presented on the table 4.6. From the table, the following were observed;

There was no significant difference between the mean T_4 levels of group 1A and group 1B. The mean T_4 level in group 1C was significantly higher than group 1D. This is an indication that iodine intake had no significant effect on mean serum T4 concentration in non-diabetic rats but significantly lowered T4 levels in diabetic rats.

There was no significant difference in the mean T_4 level of group 2A and group 2B. There was no significant difference in the mean T_4 level of group 2C and group 2D. This implies that iodine intake had no significant effect on the mean serum T4 level in non-diabetic and diabetic rats that were fed with 30% soybeans.

The mean T_4 level in group 3A was significantly higher than group 3B. The mean T_4 level in group 3C was significantly higher than group 3D. This is an indication that iodine intake significantly decreased the synthesis and secretion of T4 in non -diabetic and diabetic rats.

There was no significant difference between the mean T_4 levels of group 1A and group 2A. There was no significant difference between the mean T_4 levels of groups 1A and 3A. This shows that intake of 30% soybeans or 30% cassava had no significant effect on mean serum T4 level in non-diabetic rats in comparison to intake of normal rat feed

There was no significant difference between the mean T_4 levels in groups 1B and 2B. The mean T_4 level of group 1B was significantly higher than group 3B. This indicates that concurrent intake of iodine and 30% soybeans did not significantly alter mean serum T4 level in non-diabetic rats but concurrent iodine and 30% cassava intake significantly lowered it in comparison to intake of iodine and normal rat feed.

The mean T_4 level in group 1C was significantly lower than group 2C. The mean T_4 level of group 1C was significantly lower than group 3C. This shows that in diabetic rats, intake of 30% soybeans and intake of 30% cassava both caused a significant increase in mean serum T4 concentration in comparison to intake of

normal rat feed.

The mean T_4 level of group 1D was significantly lower than in group 2D. The mean T_4 level in group 1D was significantly lower than group 3D. This is an indication that concurrent intake of iodine and goitrogenic foods (30% cassava or soybeans) caused a significant rise in mean serum T4 level in diabetic rats in comparison to intake of normal rat feed and iodine.

Table 4.6 Mean T_4 Levels (mg/dL)

Groups	A	B	C	D
1	13.17±0.55	13.27±0.3^	11.75±0.21*	6.75±0.22
2	14.45±0.58	13.52±0.53	13.32±0.32^	6.75±0.22
3	13.08±0.16*	9.23±0.49	14.55±0.72^*	13.27±0.49^

Group 1=Normal rat feed, Group 2= 30% soybeans feed, Group 3= 30% cassava feed. (Subgroups; A= Non-diabetic, B= Non-diabetic + Iodine (10mg/kg of feed) C= Diabetic, D= Diabetic + Iodine (10mg/kg of feed)). Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA followed by students 'T test' for unpaired groups. The symbol ^ denotes $p \leq 0.05$ significantly higher in groups 1and 2 or 1and 3. The symbol *denotes $p \leq 0.05$ significantly higher in groups A and B or C and D.

The results for mean TSH (Thyroid Stimulating Hormone) are presented in table 4.7. From the table, the following were observed.

There was no significant difference between the mean TSH levels of group 1A and group 1B. The mean serum TSH level in group 1C was significantly lower than group 1D. This is an indication that iodine intake had no significant effect on mean serum TSH level in non-diabetic rats that consumed normal rat feed but significantly increased mean serum TSH concentration in diabetic rats.

There was no significant difference between the mean TSH levels of group 2A and group 2B. The mean TSH level of group 2C was significantly lower than group 2D. This shows that iodine intake had no significant effect on mean serum TSH level in non-diabetic rats that consumed 30% soybeans but significantly increased mean serum TSH concentration in diabetic rats.

The mean TSH level of group 3A was significantly lower than group 3B. There was no significant difference between the mean TSH level of group 3C and group 3D. This implies that iodine intake significantly increased mean serum TSH concentration in non-diabetic rats fed with 30% cassava but had no significant effect on mean serum TSH concentration of diabetic rats in comparison with intake of 30% cassava without iodine treatment.

There was no significant difference in the mean TSH levels of groups 1A, 2A and 3A. This is an indication that 30% soybeans intake and 30% cassava intake had no significant effect on the mean serum TSH concentration in non-diabetic rats in comparison to rats that consumed normal rat feed.

There was no significant difference between the mean TSH levels of groups 1B and 2B. The mean TSH level of group 1B was significantly lower than group 3B. This is an indication that concurrent intake of iodine and 30% soybeans had no significant effect on mean serum TSH level in non-diabetic rats but concurrent intake of iodine and 30% cassava significantly increased serum TSH level in comparison to intake of iodine and normal rat feed.

The mean TSH level of group 1C was significantly lower than group 2C. There was no significant difference between the mean TSH levels of group 1C and 3C. This implies that in diabetic rats, consumption of 30% soybeans caused significantly elevated mean serum TSH concentration but intake of 30% cassava had no significant effect on TSH concentration when compared to intake of normal rat feed.

There was no significant difference in the mean TSH levels in groups 1D and 2D. The mean TSH level in group 1D was significantly higher than group 3D. This implies that in diabetic rats, consumption of iodine and 30% soybeans did not cause any significant difference in mean serum TSH concentration but concurrent intake of iodine and 30% cassava significantly lowered mean serum TSH concentration when compared to intake of iodine and normal rat feed..

Table 4.7 Mean TSH Levels (Iμ/ml)

Groups	A	B	C	D
1	2.62±0.12	2.77±0.15	2.28±0.08	3.08±0.15^*
2	3.17±0.32	2.4±0.97	2.75±0.14^	3.12±0.05*
3	2.87±0.04	4.25±0.08^*	2.58±0.15	2.32±0.83

Group 1= Normal rat feed, Group 2= 30% soybeans feed, Group 3= 30% cassava feed. (Subgroups; A= Non-diabetic, B= Non-diabetic + Iodine (10mg/kg of feed) C= Diabetic, D= Diabetic + Iodine (10mg/kg of feed)). Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups. The symbol ^ denotes $p \leq 0.05$ significantly higher in groups 1and 2 or 1and 3. The symbol *denotes $p \leq 0.05$ significantly higher in groups A and B or C and D.

The results for antibody for thyroperoxidase (TPO) are presented in table 4.8 in

the next page. From the table, the following was observed;

The results showed negative level in all the groups and subgroups. This is an indication that iodine intake, 30% soybeans intake and 30% cassava intake did not cause the development of thyroperoxidase antibody.

Table 4.8 Antibody Index for Thyroperoxidase (TPO)

Groups	A	B	C	D

1	0.1	0.1	0.1	0.1
2	0.1	0.1	0.1	0.1
3	0.1	0.1	0.1	0.1

Group 1= Normal rat feed; Group 2= 30% soybeans feed; Group 3= 30% cassava feed. (Subgroups; A= Non-diabetic B= Non-diabetic + Iodine (10mg/kg of feed) C= Diabetic, D= Diabetic + Iodine (10mg/kg of feed)). Results are presented in Mean ±SEM. Negative: 0.1, Positive: 3.9, Calibrator: 2.9, Calibrator factor: 0.35, Cut –off: 0.6293.

The results of thyroglobulin antibody index are presented in table 4.9 in the next page. From the table, the following was observed;

The results showed negative (0.1) for thyroglobulin antibody index in all the rats. This is an indication that iodine intake, 30% soybeans and 30% cassava intake did not cause the development of thyroglobulin antibody.

Table 4.9 Antibody Index for Thyroglobulin

Groups	A	B	C	D
1	0.1	0.1	0.1	0.1
2	0.1	0.1	0.1	0.1
3	0.1	0.1	0.1	0.1

Group 1: Normal rat feed, Group 2: 30% soybeans feed, Group 3: 30% cassava feed. (Subgroups; A: Non-diabetic B: Non-diabetic + Iodine (10mg/kg of feed) C: Diabetic, D: Diabetic + Iodine (10mg/kg of feed)). Results are presented in Mean ±SEM. Negative: 0.1, Positive: 2.4, Calibrator: 2.0, Calibrator factor: 0.5, Cut- off: 0.73

4.7 Results for Oxidative Stress Markers

The results for mean serum concentrations of Malondialdehyde (MDA) are presented in table 4.10. From the table, the following were observed;

The mean serum MDA level in group 1A was significantly lower than group 1B. There was no significant difference in the mean serum MDA level of group 1C and 1D. This shows that intake of iodine caused a significant increase in mean serum level of MDA in non-diabetic rats but had no significant effect on MDA level in diabetic rats.

There was no significant difference in the mean serum MDA levels of group 2A and 2B. The mean serum MDA level in group 2C was significantly lower than group 2D. This is an indication that iodine intake had no significant effect on serum MDA level in non-diabetic rats that consumed 30% soybeans but significantly increased it in diabetic rats compared to the rats that consumed 30% soybeans without iodine treatment.

There was no significant difference in the mean serum MDA level of group 3A and 3B. There was no significant difference in the mean serum MDA levels of groups 3C and 3D. This is an indication that iodine intake had no significant effect on mean serum MDA level in both diabetic and non-diabetic rats that consumed 30% cassava in comparison to those that consumed 30% cassava without additional iodine intake.

There was no significant difference between the mean serum MDA level of groups 1A and 2A. The mean serum MDA level of group 1A was significantly lower than group 3A. This signifies that when compared to intake of normal rat feed, intake of 30% soybeans did not significantly alter mean serum MDA level but intake of 30% cassava caused significantly elevated mean serum MDA level in non-diabetic rats.

There was no significant difference in the mean MDA levels in groups 1B, 2B and 3B. This implies that concurrent intake of iodine with 30% soybeans or cassava had no significant effect on mean serum concentrations of MDA in non-diabetic

rats in comparison to intake of iodine and normal rat feed.

The mean MDA level of group 1C was significantly higher than groups 2C and 3C. This implies that in diabetic rats, consumption of 30% soybeans or 30% cassava caused significantly lowered MDA levels.

The mean MDA level of group 1D was significantly lower than group 2D and higher than group 3D. This shows that concurrent intake of iodine and 30% soybeans significantly increased the production of serum MDA. Concurrent intake of iodine and 30% cassava caused significant decrease in serum MDA level.

Table 4.10 Mean Serum Levels of MDA (µmol/l)

Groups	A	B	C	D
1	18.4±1.3	22.2±2.7*	35.2±4.7^	29.2±2.3^
2	40.4±2.4*	20.7±2.13	17.4±0.27	50.1±12.4^*
3	25.2±0.83^	22.4±2.13	18.9±0.88	21.8±1.38

Group 1= Normal rat feed; Group 2= 30% soybeans feed, Group 3= 30% cassava feed. (Subgroups; A= Non-diabetic, B= Non-diabetic + Iodine (10mg/kg of feed), C:= Diabetic, D= Diabetic + Iodine (10mg/kg of feed)). Data are expressed in mean[5] ±SEM (*n*=5). Comparisons were made using one way ANOVA and students' T test for unpaired groups. The symbol ^ denotes $p \leq 0.05$ significantly higher in groups 1and 2 or 1and 3. The symbol *denotes $p \leq 0.05$ significantly higher in groups A and B or C and D.

The results for mean Superoxide Dismutase (SOD) levels were presented in table 4.11. From the table, the following were observed;

There was no significant difference in the mean SOD levels of groups 1A and 1B. The mean SOD level of group 1C was significantly lower than group 1D. This implies that in non-diabetic rats, iodine had no significant effect on mean serum SOD level but in diabetic rats, iodine intake stimulated higher production of serum SOD.

The mean SOD level in group 2A was significantly higher than group 2B. The mean SOD level in group 2C was significantly higher than group 2D. This is an indication that in both diabetic and non-diabetic rats, concurrent consumption of iodine and 30% soybeans caused significantly lower mean serum levels of SOD.

The mean SOD level of group 3A was significantly lower than group 3B. There was no significant difference between the mean SOD levels of groups 3C and 3D. This implies that in non-diabetic rats, intake of iodine and 30% cassava caused significantly elevated serum SOD levels but had no significant effect on serum SOD levels in diabetic rats.

There was no significant difference between the mean SOD levels of groups 1A and 2A. The mean SOD level in group 1A was significantly higher than group 3A. This is an indication that in non-diabetic rats, consumption of soybeans had no significant effect on serum levels of SOD but consumption of 30% cassava caused a significant reduction in mean serum SOD level.

The mean SOD level in group 1B was significantly higher than groups 2B and 3B. This is an indication that concurrent consumption of iodine and goitrogenic foods (30% soybeans or cassava) significantly reduced mean serum SOD concentrations in non-diabetic rats.

There was no significant difference in the mean SOD levels in groups 1C, 2C and 3C. This shows that in diabetic rats, consumption of 30% soybeans or cassava did not significantly alter serum SOD levels.

The mean SOD level in group 1D was significantly higher than groups 2D and 3D. This implies that in diabetic rats, concurrent consumption of iodine and goitrogenic foods (30% soybeans and cassava) caused significantly lower levels of mean serum SOD.

Table 4.11 Mean Serum Levels of SOD (mg/ml)

Groups	A	B	C	D

1	54.06±8.74^	42.34±1.35^	44.44±2.94	68.94±0.91^*
2	45.24±8.11*	18.19±1.61	38.65±5.29*	12.04±0.17
3	16.40±1.20	31.16±2.0*	44.94±5.21	49.82±5.30

Group 1= Normal rat feed, Group 2= 30% soybeans feed, Group 3= 30% cassava feed. (Subgroups; A= Non-diabetic, B= Non-diabetic + Iodine (10mg/kg of feed), C= Diabetic, D= Diabetic + Iodine (10mg/kg of feed)). Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students' T test for unpaired groups. The symbol ^ denotes $p \leq 0.05$ significantly higher in groups 1and 2 or 1and 3. The symbol *denotes $p \leq 0.05$ significantly higher in groups A and B or C and D.

The results for the mean Nitric Oxide (NO) concentrations are presented in table 4.12. , the following were observed;

The mean NO level of group 1A was significantly higher than group 1B. There was

no significant difference between the mean NO levels of groups 1C and 1D. This is an indication that iodine intake in non-diabetic rats caused significantly reduced mean serum level of NO but had no significant effect on serum NO concentrations in diabetic rats.

The mean NO level of group 2A was significantly lower than group 2B. There was no significant difference between the mean NO levels of groups 2C and 2D. This shows that in non-diabetic rats fed with 30% soybeans, iodine intake significantly increased NO levels but had no significant effect on NO levels in diabetic rats.

There was no significant difference between the mean NO levels of groups 3A and 3B. There was no significant difference between the mean NO levels in groups 3C and 3D. This shows that in diabetic and non-diabetic rats fed with 30% cassava, iodine intake did not significantly alter the serum levels of NO.

The mean NO level in group 1A was significantly higher than group 2A and lower than group 3A. This is an indication in non-diabetic rats, consumption of 30% soybeans caused lower NO levels and consumption of 30% cassava caused higher mean serum NO levels.

The mean NO level in group 1B was significantly lower than group 2B. There was no significant difference in the mean NO level in groups 1B and 2B. This shows that in non-diabetic rats, concurrent consumption of iodine and 30% soybeans significantly increased NO levels but consumption of iodine and 30% cassava did not significantly alter mean serum NO concentration.

The mean NO level in group 1C was significantly lower than group 2C. There was no significant difference in the mean NO levels in groups 1C and 3C. This shows that in diabetic rats, intake of 30% soybeans significantly increased serum NO production but 30% cassava intake had no significant effect on NO levels.

There was no significant difference in the mean NO levels in groups 1D, 2D and 3D. This implies that concurrent iodine and goitrogenic food (30% soybeans or cassava) intake did not significantly alter mean serum NO concentration in comparison to intake of normal rat feed and iodine.

Table 4.12 Results for NO (µm/l)

Groups	A	B	C	D
1	14.08±0.38^*	13.24±0.07	14.56±0.08	15.03±0.36
2	9.37±0.08	15.66±0.8^*	14.92±0.08^	15.96±0.65
3	17.07±0.92^	12.44±1.98	15.11±0.65	14.04±0.80

Group 1= Normal rat feed, Group 2= 30% soybeans feed, Group 3= 30% cassava feed. (Subgroups; A= Non-diabetic, B= Non-diabetic + Iodine (10mg/kg of feed), C= Diabetic, D= Diabetic + Iodine (10mg/kg of feed)). Data are expressed in mean ±SEM (*n*=5). Comparisons were made using one way ANOVA and students 'T test' for unpaired groups. The symbol ^ denotes $p \leq 0.05$ significantly higher in groups 1and 2 or 1and 3. The symbol *denotes $p \leq 0.05$ significantly higher in groups A and B or C and D.

CHAPTER FIVE

Discussion

5.0. This chapter discusses the results presented in the previous chapter

5.1 Rat Feed Analysis

The iodine content observed from the rat feeds showed that based on the daily iodine needs of the rats estimated by Halverson *et al.* (1949), the standard rat feed, 30% *G.max* (soybeans) feed and 30% *M. esculenta* (cassava) feed all had adequate iodine contents. This is in line with the findings of Salau *et al.,* (2008) who concluded that consumption of 200 g edible portion *of G. max* or *M.esculenta* would yield more than 200 µg iodine, which is the required dietary allowance (CDA, 1980) of an adult. Although the iodine content of the food cannot be taken as absolute because the iodine content of plant is also a function of the soil content (Babara, 1994) and the availability of the iodine is dependent on other inherent constituents of the plants such as goitrogens in *Manihot esculenta* and *Glycine max* as well as processing method (Salau *et al*., 2008).

The lower caloric content observed in cassava when compared to the normal rat feed is probably because cassava although rich in carbohydrate has high fibre content. The higher caloric content of soybeans in comparison to cassava may be attributed the fact that it is rich in protein, carbohydrates and fat (US soybean export council, USSEC, 2015) and because fibre content was reduced as a result of it been dehuled. Wetter *et al.,* (1999) observed that caloric restriction did not reduce invivo glucose uptake by tissues and increased it in several insulin-sensitive tissues. Furthermore, they observed significant reduction in insulinaemia which was previously observed by (Dean *et al*. 1998) who also observed a reduction in C-peptide (an indicator of insulin secretion) and significant reduction in epididymal and retroperitoneal fat pad

masses. From these findings, it can be inferred that caloric restriction is important in controlling type II diabetes mellitus.

5.2 Glucose Homeostasis

Walter and Mclean (1967) earlier observed decreased activities of hexokinase in hypothyroid rats and this could explain the findings of Sudipta *et al* (2007) who observed elevated blood glucose level even after 24 hours of glucose loading in hypothyroid rats. In hyperthyroid humans as well as in experimental thyrotoxicosis in animals, glucose turnover and hepatic glucose production are increased due to increased metabolic rate and peripheral glucose utilization (Menahan and Weiland, 1969). This was also reported by Dimitriadis *et al.,* (1985) who observed that experimental and spontaneous hyperthyroidism in humans caused increased glucose production and impaired suppression of glucose production by insulin.

According to Derg (2009), in inflammatory diseases, relations between oxidative stress and insulin resistance, each of them triggers or enhances the other one. The significantly higher mean blood glucose levels after 30 minutes of glucose ingestion in the oral glucose tolerance test and the significantly higher fasting blood glucose level observed in non-diabetic rats fed with normal rat feed and iodine supplement could be linked to the increase in oxidative stress (increased MDA levels and decreased NO levels) and lower mean fasting serum insulin levels observed. The significantly lower insulin levels could be due to some action of iodine in suppressing pancreatic secretion of insulin when consumed with normal rat feed.

According to Sudipta *et al*., (2007) and Arigi *et al*., (2014), hypothyroidism disrupts glucose homeostasis and fasting blood glucose level. This is observed in the diabetic rats fed with normal rat feed and iodine because it was observed that there was significantly elevated mean blood glucose levels throughout the oral glucose tolerance test and in the first 30 minutes of the insulin tolerance test.

According to Kwon *et al*., (2010) 'Historically, the incidence of type 2 diabetes has been lower in Asian populations compared with those in Western

countries. One possible reason for the lower incidence among Asians is that they consume fermented soybean products, which are unique to the traditional Asian diet'. Contrary to the findings of Kwon *et al*. a higher blood glucose level was observed 90 and 120 minutes after insulin injection in the insulin tolerance test and significantly higher fasting mean serum insulin level was observed in non-diabetic rats. This may be due to significantly lower mean serum NO concentration observed and it is in line with the findings of Shankar *et al*., (1998), who reported that systemic inhibition of NO Synthase with N^G monomethyl-L-arginine caused acute insulin resistance but the mechanism by which it caused insulin resistance was unknown. Iodine intake had no significant effect on fasting blood glucose level and insulin concentration in comparison to intake of 30% soybeans in non-diabetic rats.

The result observed in diabetic rats was contrary to the findings of Lu *et al*., (2012) who reported that aglycin; a soybean peptide improved oral glucose tolerance in streptozotocin-induced diabetic rats. Significantly higher mean fasting blood glucose and blood glucose levels observed during oral glucose tolerance and insulin tolerance tests could be as a result of the significantly lower mean serum insulin levels observed. This is contrary to the findings of Lu *et al.,* (2012) probably because they experimented with type II diabetes mellitus. Weight loss in diabetics is a result of muscle wasting and loss of fat pads and a significantly higher weight loss is an indication of more severe diabetes mellitus which was observed in the soybeans-fed diabetic rats. This is in line with the findings of Wetter *et al.,* (1999) that caloric restriction caused significant reduction in insulinaemia which was previously observed by Dean *et al*., (1998) who also observed a reduction in C-peptide (an indicator of insulin secretion) and significant reduction in epididymal and retroperitoneal fat pad masses.. With iodine supplement, the significantly higher fasting blood glucose level and significantly increased insulin level in diabetic rats is not well understood as lower mean blood glucose levels were observed throughout the insulin tolerance test. Excess iodine intake probably increased insulin requirements in diabetic rats that consumed 30% soybeans.

Swai *et al*., (1992) concluded that high dietary cyanide exposure was not found to have had a significant effect on the prevalence of diabetes in an

undernourished population and Yessoufou *et al.,* (2006) reported that cyanide-free cassava did not induce diabetes in non-diabetic rats. In spite of this, significantly higher fasting blood glucose levels and significantly lower mean serum levels of insulin were observed in non-diabetic rats fed with 30% cassava feed when compared with the non-diabetic rats fed with normal rat feed although there was no significant effect on glucose homeostasis. This might be due to the increase in oxidative stress markers observed in these rats. The opposite was observed with concurrent iodine intake which caused significantly higher insulin levels and significantly lower fasting blood glucose levels. This might be an indication that iodine intake had anti-hyperglycaemic properties in rats that consumed 30% cassava.

Yessoufou *et al.,* (2006) reported that diabetic rats fed with cyanide-free cassava had aggravated hyperglycaemia. This could be the reason for the observed insulin resistance in type I diabetic rats fed with 30% cassava feed. The development of insulin resistance may be due to significantly higher T_4 levels observed as hyperthyroidism can lead to a dysfunction in glucose homeostasis. Iodine intake caused lower serum insulin levels and had no significant effect on fasting blood glucose levels when compared to diabetic rats that consumed normal rat feed. This is a further indicator of anti-hyperglycaemic effects of iodine intake in 30% cassava-fed rats which was earlier observed in the non-diabetic rats.

5.3 Body Weight Changes

One of the symptoms of hypothyroidism is weight gain as a result of a reduction in metabolic rates. The significantly higher mean body weight gain observed after 8 weeks in diabetic rats that consumed normal rat feed and iodine supplement is most likely due to observed hypothyroidism (significantly lower mean serum T_4 levels and significantly elevated mean serum TSH levels) in these experimental rats.

Thyroid hormones are essential in both specific and general growth (Hall, 2008). The significantly lower weight gain observed in soybean and iodine fed non-diabetic rats after 6 weeks of the experiment could be due to hypothyroidism as the rats were still at a growing stage in the first 6 weeks of

the experiment and there was no significant difference in weight gain after 8 weeks of the experiment.

Dean *et al.*, (1998) observed significant reduction in epididymal and retroperitoneal fat pad masses in diabetic rats. In diabetic rats fed with soybeans, the significantly higher weight loss observed after induction of diabetes mellitus could be due to loss of muscle mass as mean blood glucose levels were more elevated than in rats fed with normal rat feed. Greatly increased thyroid hormone levels almost always decreases the body weight (Hall, 2008). Higher weight loss observed could also be due to increase in thyroid hormone (T_3) levels observed in this group.

Weight loss observed in iodine and soybeans-fed diabetic rats when compared to the diabetic rats that consumed normal rat feed and iodine could also be due to the significantly higher blood glucose levels observed in the soybeans and iodine fed-rats.

In non-diabetic rats fed with 30% cassava, weight gain which was observed with iodine intake could be due to suppressed thyroid function observed in this group (significantly lower T_4 levels and significantly higher TSH levels).

In diabetic rats that consumed 30% cassava, the significantly higher weight gain observed in comparison to diabetic rats that consumed normal rat feed might be due to lower mean blood glucose levels observed which is an indication that diabetes mellitus in the cassava-fed group was not as severe as that of the rats fed with normal rat feed and therefore had less effect on weight loss in the diabetic rats.

5.4 Thyroid Function

Wolff and Chaikoff (1948) reported that injection of iodine in rats almost completely inhibited organification (oxidation of iodide) in the thyroid gland which lasted for about ten days and was followed by an “escape phenomenon” which is described by adaption and resumption of the normal organification of iodine and normal thyroid peroxidise function. Kovac *et al.*, (2002) observed the effects of iodine toxicity in dairy cows as tachycardia, nervousness, loss of weight, and a high level of metabolism. Katagiri *et al.*, (2017) observed that

chronic exposure to excess iodine from water or poorly monitored salts are risk factors for hypothyroidism in free-living populations.

The observed results is in line with the findings of Wolf and Chaikoff (1948) since the duration of the experiment was more than ten days which was the duration observed by them for the 'escape phenomenon'. In addition, the absence of thyroid autoimmune antibodies thyroglobulin antibody (TgAb) and thyroid peroxidise antibody (TPOAb) is an indication that iodine intake had no significant effect on the thyroid function in non-diabetic rats fed with normal rat feed.

Maradi *et al*., (2001) concluded that there is decreased iodine uptake by diabetic serum. According to them, the cause for decreased iodine uptake may be related to high blood glucose level which possibly may be causing some alteration in the structure of bio-molecules by glycation leading to decrease in the binding sites of iodine. Regardless, the iodine uptake in the diabetic rats that consumed iodine with their feed at a concentration of 10mg/kg of feed would be significantly higher than the diabetic rats that did not receive iodine but the diabetic state of the rats might have prevented them from adapting to high iodine intake. The significantly lower mean serum T_3 and T_4 levels observed in diabetic rats that received iodine which was significantly lower than the diabetic rats that did not receive iodine might be due to the combination of excess iodine consumption and diabetes mellitus condition. Lower levels of thyroid hormones stimulate the anterior pituitary to secrete more TSH (Guyton and Hall, 2008) therefore, significantly lower T_3 and T_4 levels in group 1D would be the stimulus for increased TSH secretion. Thyroglobulin Antibody (TGAb) and Thyroid Peroxidise Antibody (TPOAb) were observed by Lindberg (1997) and Otken *et al.,* (2006) in type I diabetic patients but these were not observed in this experiment.

Block and Anderson (1961) concluded that soybean had a goitrogenic property which is removed and destroyed during processing. Ikeda *et al*., (2000) observed that defatted soybean had no significant effect on thyroid weight and T_4 levels but significantly increased serum TSH levels, in 2001, Ikeda *et al*. concluded that soybean intake specifically interacts with iodine deficiency in

induction of thyroid proliferative lesions in rats only at high doses. The soybeans used for this experiment were well processed which could be the reason why significantly higher levels of T_3 were observed in the 30% soybeans -fed rats when compared to rats that fed on normal rat feed. Higher T_3 levels could be an indication that soybeans consumption increased the T_3 requirement because the mean serum levels of T_4 and TSH were not significantly affected and there was no significant difference in weight gain in the growing rats (after 6 weeks). Consumption of 30% soybeans did not result in autoimmune thyroid disorders as the markers of autoimmune thyroid disorders (TPOAb and TgAb) were absent neither did it significantly affect weight gain.

Block and Anderson (1961) reported a curative action of iodine at a dose of 160ug/100g on soybean-induced goitre in rats and concluded that soybean had a goitrogenic property which is removed and destroyed during processing. Messina *et al.,* (2006) concluded that soy food consumers should make sure their intake of iodine is adequate. The concentration of iodine in the diets of the rats used in this experiment was much higher (estimated 1.1mg/100g). Therefore, significantly lower mean serum T_3 level demonstrates the effect of iodine intake at estimated 1.1mg/100g or 0.5mg of iodine per rat per day for 8 weeks in suppressing thyroid function in rats fed with 30% soybeans. Although the serum T_3 levels in the iodine-fed rats was not significantly different from that of the rats that consumed normal rat feed, the significantly lower serum T_3 levels could be the reason for the significantly lower weight gain observed in the first six weeks of the experiment which is an indication of reduced growth rate because the rats were at an adolescent stage when the experiment commenced as there was no significant difference in weight gained after 8weeks. This further showed that soybeans consumption probably increased T_3 requirement in non-diabetic rats.

Panda *et al*., (2009), concluded that their findings suggest that soy sterols, at a moderate concentration potentially ameliorates hyperthyroidism and diabetes, but at higher concentration it may exert adverse effects. These ‘adverse effects’ were observed in this experiment as the levels of T_4 and TSH were

significantly higher than diabetic rats that consumed normal rat feed and there was a significantly higher weight loss although there was no significant difference in the mean serum level of T_3. The results observed with iodine intake is an indication that higher serum iodine concentrations probably stimulated the thyrotropes in the anterior pituitary to synthesise and secrete more TSH or it stimulated the hypothalamus to secrete more TRH although the T_3 and T_4 levels were not significantly affected and there was absence of TgAb and TPOAb. It is not properly understood why iodine supplement increased TSH concentration.

A report by Chandra *et al.*, (2006) on an experiment which lasted for 90 days stated that 'After consumption, cyanogenic constituents in cassava are metabolized to thiocyanate and iodine-retaining capacity seems to be dependent on thiocyanate exposure. In cassava-fed rats, the urinary iodine concentration resembled the state of adequate iodine nutritive but thyroid gland fails to utilize available iodine. Increased thyroid weight ($P < 0.001$), inhibited thyroid peroxidase (TPO) activity and reduced thyroid hormone profiles ($P < 0.001$) were noted in fresh and cooked cassava-fed rats, resembling a relative state of morphological as well as biochemical hypothyroidism even in presence of adequate iodine'. Mlingi *et al.,* (1996) stated that cyanide exposure from consumption of insufficiently processed cassava has been implicated in aggravating iodine deficiency disorders (IDD). The cyanide metabolite, thiocyanate may interfere with iodine uptake of the thyroid gland.

The retention of cyanide in garri is only 1.8-2.4 % (Cardoso *et al.*, 2005). Cassava that was used for the experiment was processed into garri by crushing, squeezing out the water, fermenting for 3days and roasted. Therefore, the goitrogenic effect of cassava was not manifested in non-diabetic probably because it was well-processed to reduce cyanide content to the minimum. Therefore, it had no significant efffect on thyroid function (the mean serum levels of T_3, T_4 and TSH were not significantly affected). There was also absence of autoimmune antibodies TgAb and TPOAb. Hence, there was no significant difference in the weight gain over the course of the

experiment.

In non-diabetic rats that consumed 30% cassava and iodine, thyroid function significantly reduced as observed in the mean serum T_3 and T_4 levels which were significantly lower and TSH level which was significantly higher. This infers that the consumption of cassava prevented the thyroids of the rats from recovery from excess iodine intake and this could be due to the small traces of cyanide in cassava or probably some other constituent of cassava.

According to Maradi *et al.*, (2001), there is decreased iodine uptake by diabetic serum and the cause for decreased iodine uptake may be related to high blood sugar level which possibly may be causing some alteration in the structure of biomolecules by glycation leading to decrease in the binding sites of iodine. The significantly higher mean serum level of T_4 observed in diabetic rats fed with 30% cassava diet when compared to diabetic rats that consumed standard rat feed might be due to the fact that the mean blood glucose levels of the diabetic rats fed with standard rat feed was significantly higher than those fed with 30% cassava feed and iodine is essential in thyroid hormone synthesis. The significantly higher weight gain observed after 6 weeks and 8 weeks might be due to its effect on glucose homeostasis that was observed. Iodine intake in diabetic rats fed with 30% cassava caused a significantly lower T_4 concentration although it had no significant effect on T_3 and TSH mean serum levels and weight gain after 6 and 8weeks. This may be due to the iodine toxicity stated by Katagiri *et al.*, (2017).

5.5 Oxidative Stress Markers

Malondialdehyde (MDA) levels have been showed to increase with hypothyroidism (Dumitriu *et al.*, 1988, Cheserek *et al.*, 2015, Mancini *et al.*, 2016, Chakrabarti *et al.*, 2016) and hyperthyroidism (Dumitriu *et al.*, 1988, Chakrabarti *et al.*, 2016). In non-diabetic rats fed with normal rat feed, although there was no significant difference in thyroid hormone levels, the significantly higher MDA and lower Nitric Oxide (NO) levels with iodine supplement is an indication that iodine supplement induced oxidative stress in rats fed with normal rat feed through a mechanism that does not involve thyroid function although there was no significant difference in superoxide dismutase (SOD)

levels.

Verma *et al*., (1991) observed that administration of TSH caused a dramatic loss in SOD in the adrenal gland. The significantly higher levels of TSH in the diabetic rats might be the cause of the significantly lower serum SOD levels although MDA and NO levels were not significantly affected. This is an indication that iodine supplement in diabetic rats fed with normal rat feed increased oxidative stress by reducing SOD levels.

In 2005, Engleman *et al.* observed that soy protein had no significant effect on reducing oxidative damage or favourably altering blood lipids in postmenopausal women. Panda *et al*., (2009) reported that soy sterol caused an increase in superoxide dismutase, catalase and reduced glutathione. Georgetti *et al.,* (2013) also reported that fermented soybean dried reduced 12-0-tetradecanoylphorbol-13-acetate-(TPA) induced oxidative stress in skin of hairless mice. Teixeira *et al*., (2014) stated that soy isoflavones improved antioxidant status and counteracted oxidative stress in the uterus of ovariectomized rats. The significantly lower mean serum level of NO suggests soybeans increased oxidative stress which is contrary to the findings of Panda *et al*., (2009) , Georgetti *et al.,* (2013) and Texiera *et al*., (2014) which could probably be because the antioxidant properties present in soybeans could be in the peels. The elevated NO levels that was observed in the iodine-fed rats is contrary to the observations of Quesada *et al*., (2002) who reported increased NO synthase levels in hyperthyroidism . This could be due to a substance present in soybean or iodine or an interraction between a substance in soybeans and iodine because in the rats fed with normal rat feed, iodine supplement significantly reduced NO levels. The reason for the significantly lower mean serum SOD levels observed with iodine supplement is not known although there was no significant difference in mean serum MDA levels.

From the results, it was observed that soybean reduced oxidative stress in diabetic rats because the mean serum levels of MDA were lower and NO was higher although there was no significant difference in SOD levels. This might be due to its effect in improving thyroid function in diabetic rats as hypothyroidism is linked to higher levels of MDA (Cheserek *et al.,* 2015 and

Chakrabarti *et al*., 2016). The significantly lower SOD levels observed in the iodine-supplemented rats could be due to increased TSH as reported by Verma *et al*., (1991). The lower SOD levels coupled with significantly higher MDA levels indicate an increase in oxidative stress with iodine supplement although it had no effect on mean serum NO concentration.

Bahekar and Kale (2016) concluded that cassava leaves significantly increased the serum SOD levels in wistar rats but had no significant effect on MDA levels but this was not observed with cassava tubers as it showed an ability to increase oxidative stress by increasing MDA level and significantly reducing SOD although it significantly increased NO levels. The significantly higher mean serum SOD levels observed with iodine supplement is an indication that iodine with cassava feed reduced oxidative stress although there was no significant difference in NO and MDA levels.

Bahekar and Kale (2016) concluded that "*Manihot esculenta* leaf extract did not exhibit antioxidant activity in terms of MDA level reduction. However, it significantly increased serum levels of the antioxidant enzymes (SOD, GSH, and CAT) exerting a potent antioxidant effect in a graded manner". It was observed that cassava-rich diet significantly reduced oxidative stress in diabetic rats because lower mean serum MDA levels were observed compared to rats fed with normal rat feed although it had no significant effect on SOD and NO levels. The results of the mean serum levels of MDA, SOD and NO which showed no significant difference with iodine supplement, indicated that iodine had no significant effect on oxidative stress in diabetic rats fed with 30% cassava.

CHAPTER SIX

6.0 Summary and Conclusions

This chapter gives a summary of the results and discussions that were earlier presented in the previous chapters

6.1 Findings

The iodine content of normal rat feed, cassava and soybeans was adequate for the recommended daily intake. Cassava had the lowest calories when compared with soybeans and normal rat feed.

Excessive iodine intake significantly suppressed thyroid function in diabetic rats that consumed normal rat feed, in non-diabetic rats fed with 30% soybeans and in diabetic and non-diabetic rats fed with 30% cassava.

Excessive iodine intake significantly altered glucose homeostasis by causing increased mean blood glucose levels in diabetic rats that consumed normal rat feed.

Excess iodine and 30% soybeans intake significantly increased fasting blood glucose levels and altered insulin levels in non-diabetic and diabetic rats.

30% soybeans consumption increased blood glucose levels in non-diabetic and diabetic rats by altering insulin levels.

30% cassava feed caused insulin resistance in diabetic rats and lowered insulin levels in non-diabetic rats.

Excessive iodine intake, 30% soybeans intake and 30% cassava intake all increased oxidative stress.

6.2 Conclusions

Excess iodine intake caused suppressed thyroid function and significantly increased hyperglycaemia in diabetic wistar rats.

Excess iodine and concurrent goitrogenic food intake suppressed thyroid function, increased mean blood glucose levels and oxidative stress in diabetic and non-diabetic wistar rats.

6.3 Recommendations

Excessive iodine intake and exposure should be avoided.

Goitrogenic foods consumption should be curtailed by finding more healthy alternative sources of nutrients.

Soybeans and cassava should be properly processed before consumption

6.4 Contributions to Knowledge

This study has shed light on the following;

1. Excess iodine intake can lead to hyperglycaemia in non-diabetic rats and increase hyperglycaemia in diabetic rats
2. Contrary to earlier studies which postulated that consuming goitrogenic foods required additional iodine intake, this study showed that concurrent intake of excess iodine and goitrogenic foods significantly reduced thyroid function.
3. Consumption of properly processed goitrogenic foods did not significantly alter thyroid function.

6.5 Further studies

Although the study has revealed the hyperglycaemic effect of excess iodine and goitrogenic food intake, further investigations that may be carried out in this area include;

1. Determining the manner in which iodine might affect insulin synthesis and secretion.
2. Investigating the iodine contents of different forms of processed foods with iodine additives and the safety in the consumption of these foods.
3. Research on the inherent dangers of indiscriminate addition of soybeans and cassava to a wide range of processed foods.

REFERENCES

A.O.A.C (1984)- Association of official Analytical Chemist's Official methods of Analysis. 14th Edition. Arlington U.S.A, 33. 143-33. 147.

Akinola O, Gabriel M, Suleiman A and Olorunsogbon F (2012). Treatment of Alloxan-Induced Diabetic Rats with Metformin or Glitazones is Associated with Amelioration of Hyperglycaemia and Neuroprotection. *The Open Diabetes Journal*, 5, 8-12

Albert A, Keating FR Jr. (1949). Metabolic studies with I131 labeled thyroid compounds*. J Clin Endocrinol* 9:1406–1421.

Alvino CG, Acquaviva AM, Catanzano AM, Tassi V (1995). Evidence that Thyroglobulin has an Associated Protein Kinase Activity Correlated with the Presence of an Adenosine Triphosphate Binding Site.*Endocrinology.* 136:3179-3185.

American Association of Clinical Endocrinologists (2009).Endocrinology Practical.

Arigi, Q. E, Fabiyi, T.D and Fasanmade, A.A (2014). Thyroidectomy and Thyroxine Replacement Caused Impaired Oral Glucose Tolerance in Rat *World Journal of Medical Sciences*. Vol11 (3): 348-352

ATSDR (2004). Assessment of joint toxic action of chemical mixtures.

Bahekar S.E and Kale R.S (2016).Evaluation of Antioxidant Activity of Manihot

esculenta crantz in Wistar Rats*. Journal of Pharmaceutical and Bioallied Sciences*. Apr-Jun;8(2): 199-123. Doi: 10.4103/0975-7406.171697. PMCID: PMC4832901

Baran DT, Braverman LE (1991). Thyroid hormones and bone mass. *J Clin Endocrinol Metab* 72:1182–1183.

Barbara U (1994). Current Status of Iodine Deficiency Disorders. A global perspective; NU News on Health Care in Developing Countries.3/94(8): 4 -7.

Baundry N, Lejeune P.J, Delom F, et al (1998). Role of multimerized porcine thyroglobulin in iodine storage.*Biochem Biophys Res Comm* 242:292-296.

Benmiloud M, Bachtarazat H, Chaoki MB (1983). Public health and nutritional aspect of goiter and cretinism in Africa. In: Delange F, Ahluwalia R (eds) Cassava toxicity and thyroid. Research and public health issue. IDRC, p. 50.

Bernal J (1999).Iodine and brain development.*Biofactors* 10:271–276.

Berndorfer U, Wilms H, Herzog V (1996). Multimerization of thyroglobulin (TG) during extracellular storage: isolation of highly cross-linked TG from human thyroids. *J Clin Endocrinol Metab.* 81:1918-1926.

Berson SA, Yalow RS (1955).The iodide trapping and binding functions of the thyroid. *J Clin Invest* 34:186.

Bhat MK, Ashizawa K, Cheng SY (1994). Phosphorylation enhances the target gene sequence-dependent dimerization of thyroid hormone receptor with retinoid X receptor. *Proc Natl Acad Sci .* 91:7927–7931.

Bidart JM, Mian C, Lazar V, Russo D, Filetti S, Caillou B, Schlumberger M (2000). Expression of pendrin and the Pendred syndrome (PDS) gene in human thyroid tissues*. J Clin Endocrinol Metab* 85:2028-2033.

Blanco JC, Minucci S, Lu J, Yang XJ, Walker KK, Chen H, Evans RM, Nakatani Y,

Ozato K(1998). The histone acetylase PCAF is a nuclear receptor coactivator. *Genes Dev* 12:1638–1651.

Boelen, A. (2009). Thyroid Hormones and Glucose Metabolism: the story begins before birth. The Physiological Society.

Bowen R (1999). Chemistry Of Thyroid Hormones. Index Of Thyroid And Partathyroid Hormones. Retrieved 25 of March 2012.

Braverman, L.E (1994). Iodine and the Thyroid-33 years of study.*Thyroid*, 4: 351 -56

Brenta G (2010). Diabetes and Thyroid Disorders.*Br. J. of Diab.and Vasc. Dis*. 10(4):172

British Thyroid Foundation (2006)

Bureau of Medicine And Surgery (2001)

Cardoso, P.A, Mirione, E, Ernesto, M, Massaza,F, Cliff, J, Haque, M.R, Bradbury, J.H (2005). Processing of Cassava Roots to remove Cyanogens.*Journal of Food Composition and Analysis*. 18(2005)451-60

Carre JL, Demerens C, Rodriguez-Pena A, Floch HH, Vincendon G, Sarlieve LL (1998). Thyroid hormone receptor isoforms are sequentially expressed in oligodendrocyte lineage cells during rat cerebral development. *J Neurosci Res* 54:584–594.

Chakrabarti, S.K, Ghosoh, S, Banerjee, S, Mukherjee, S and Chowdhury, S (2016).Oxidative Stress in Hypothyroid Patients and the Role of Antioxidant Supplementation.*Indian Journal of Endocrinology and Metabolism*. 20(5): 674-78. Doi: 10.4103/2230-8210.190555. PMCID: PMC5040049

Cheserek, M.J, Wu, G, Ntazinda, A, Shi, Y, Shen, L and Le, G (2015). Association Between Thyroid Hormones, Lipids and Oxidative Stress Markers in Subclinical Hypothyroidism. *Journal of Medical Biochemistry*. 34(3): 323 -331. Doi.10.2478/jomb-2014-0044. PMCID:PMC4922350.

Clark D, Sokoloff, L. (1999). Basic Neurochemistry: Molecular, Cellular and Medical Aspects. Lippincott. pp. 637–670.

Cochaux P, Van Sande J, Dumont JE (1982).Inhibition of the cyclic AMP-adenylate cyclase system and of secretion by high concentrations of adenosine in the dog thyroid. *Biochem Pharmacol* 31:3763-3767.

Committee on Dietary Allowance, Food and Nutrition Board (1980). Recommended dietary allowance. 18th revised ed. National Academy of Sciences, Washington DC, pp. 53-54

Consiglio E, Salvatore G, Rall JE, Kohn LD (1979). Thyroglobulin interactions with thyroid membranes.The existence of specific receptors and their potential role.*J Biol Chem* 254:5065-5076.

Cooper G (2007). The Cell, American Society of Microbiology, p 72

Cooper RA (2007). Iodine revisited. *Int Wound J*. 4(2): 124-37

Corpeleijn E, Mensink M, Kooi ME, Roekaerts PM, Saris WH, Blaak EE (2008). Impaired skeletal muscle substrate oxidation in glucose-intolerant men improves after weight loss. *Obesity (Silver Spring)*.16(5):1025-32. [Medline].

Corvilain B, Collyn L, van Sande J, Dumont JE (2000). Stimulation by iodide of H2O2 generation in thyroid slices from several species. *Am J Physiol Endocrinol Metab*. 278:692-699.

Corvilain B, Laurent E, Lecomte M, van Sande J, Dumont JE (1994). Role of the cyclic adenosine 3',5'-monophosphate and the phosphatidylinositol-Ca^{2+} cascades in mediating the effects of thyrotropin and iodide on hormone synthesis and secretion in human thyroid slices. *J Clin Endocrinol Metab* 79:152-159.

Corvilain B, Van Sande J, Dumont JE (1988). Inhibition by iodide of iodide binding to proteins: the "Wolff-Chaikoff" effect is caused by inhibition of H_2O_2 generation. *Biochem Biophys Res Commun.* 154:1287-1292.

Cramer W, M'Call R (1918) Carbohydrate Metabolism In Relation To The Thyroid Gland III. The Effect Of Thyroidectomy In Rats On The Gaseous Metabolism.*Experi. Physiol.* 12, 81-95

Czarnocka B, Ruf J, Ferrand M, Carayon P, Lissitzky S (1985). Purification of the human thyroid peroxidase and its identification as the microsomal antigen involved in autoimmune thyroid diseases.*FEBS Lett.* 190:147-152.

Dagogo-Jack S, Santiago JV (1997).Pathophysiology of type 2 diabetes and modes of action of therapeutic interventions.*Arch Intern Med.*157(16):1802-17. [Medline].

Damante G, Di Lauro R (1994). Thyroid-specific gene expression. Biochim Biophys Acta 1218:255-266.

Davidson NO, Carlos RC, Lukaszewicz AM (1990). Apolipoprotein B mRNA editing is modulated by thyroid hormone analogs but not growth hormone administration in the rat. *Mol Endocrinol* 4:779–785.

Dean, D. T, Bronzinick, J. T, Cushman, S. W and Cantee, G. D (1998). Calorie Restriction Increases Cell surface GLUT-4 in Insulin Stimulated Skeletal Muscle. *American Journal of Physiology, Endocrinology and Metabolism.* 275(6) E957-E958.

DeFronzo RA (2004). Pathogenesis of type 2 diabetes mellitus.*Med Clin North Am.* 88(4):787-835, ix. [Medline].

DeGroot LJ (1966). Kinetic analysis of iodine metabolism.*J Clin Endocrinol Metab.* 26:149.dihydroiodide and sodium or potassium iodide by dairy cows. J. Dairy Sci., 56, 378–384.

Derg, A. K (2009). Insulin Resistance Is Related to Oxidative Stress in Systemic Lupus Erythematosis. *National Library of Medicine*. Feb; 9 (1) 23. PMID 19196569

Dimitriadis G, Baker, B, Marsh, H, Mandriano, L et al. (1988). Effect of Thyroid Hormone excess on Action, Secretion and Metabolism of Insulin in

Humans.*Am. J. Physiol. Endo* 248 5 pp593-601

Dohan O, De la Vieja A, Paroder V, Riedel C, Artani M, Reed M, Ginter CS, Carrasco N (2003). The sodium/iodide symporter (NIS): characterization, regulation, and medical significance. *Endocr Rev.* 24:48-77.

Dumitriu, L, Bartoc, R, Ursu, H, Purice, M and Ionescu, V (1988).Significance of High Levels of Serum Malonyldialdehyde (MDA) and Ceruloplasmin (CP) in Hyper- and Hypothyroidism.*Endocrinologie*.26 (1):35-8

Dunn AD, Crutchfield HE, Dunn JT (1991). Thyroglobulin processing by thyroidal proteases.*J Biol Chem* 266:20198-20204.

Dunn AD, Dunn JT (1988). Cysteine proteinasee from human thyroid and their actions on thyroglobulin.*Endocrinology* 123:1089-1097.

Dunn JT, Dunn AD (2000). Thyroglobulin: chemistry, biosynthesis, and proteolysis. In: The Thyroid, 8th edition. Eds: Braverman LE, Utiger R. Lippincott Williams & Wilkins, Philadelphia , pp 91-104.

Dunn JT, Kim PS, Dunn AD (1982). Favored sites for thyroid hormone formation on the peptide chains of human thyroglobulin. J Biol Chem 257:88-94.

Dupuy C, Virion A, Ohayon R, et al (1991). Mechanism of hydrogen peroxide formation catalyzed by NADPH oxidase in thyroid plasma membrane.*J Biol Chem.* 266:3739-3743

Ekholm R (1981). Iodination of thyroglobulin.An intracellular or extracellular process?*Mol Cell Endocrinol* 24:141-163.

Elliot.B (2017). Cassava: Benefits and Dangers. Authority Nutrition.*Healthline* newsletter.

FAO/WHO (2002). Human Vitamin and Mineral Requirements. Report of a Joint FAO/WHO Expert Consultation.

Ferrand M, Le Fourn V, Franc JL (2003). Increasing diversity of human

thyroperoxidase generated by alternative splicing, characterized by molecular cloning of new transcripts with single- and multispliced mRNAs. *J Biol Chem* 278:3793-3800.

Fonlupt P, Audebet C, Gire V, Bernier-Valentin F, Rousset B (1997). Calcium is transported into the lumen of pig thyroid follicles by fluid phase basolateral to apical transcytosis. *J Cell Physiol* 171:43-51.

Fred W. S (2006). Glucose and Glucose-Containing Syrups" in Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH, Weinheim.doi: 10.1002/14356007.a12_457.pub2

Gaitan E (1989). Environmental Goitrogenesis. Boca Raton: CRC Press.

Gardner DF, Utiger RD, Schwartz SL, Witorsch P, Myers B, Braverman LA, Witorsch RJ (1987). Effects of oral erythrosine (2',4',5',7'-tetraiodofluorescein) on thyroid function in normal men. *Toxicol Appl Pharmacol* 91:299-304.

Gartner R, Dugrillon A, Bechtner G (1996). Iodolipids and thyroid function and growth. In: The Thyroid and Iodine. Eds: Nauman J, Glinoer D, Braverman LE, Hostalek U. Merck European Thyroid Symposium, Stuttgart, New York. pp 19-27

Georgetti, S. R, Casangrande, R, Vicentini F.T. M. C, Baracat, M. M, Verri, W. A and Fonseca, M. J. V (2013). Protective Effect of Fermented Soybean Dried Extracts Against TPA-Induced Oxidative Stress in Hairless Mice Skin. *Biomed Research International.* Vol. 2013, Article ID 340626. Pg 8

Ghent WR, Eskin BA, Low DA, Hill LP (1993).Iodine replacement in fibrocystic disease of the breast.*Can J Surg*. 36(5):453-60

Ghent WR, Eskin BA, Low DA, Hill LP. 1993. Iodine replacement in fibrocystic

Guyton A.C, Hall, J. E (2006).Textbook of Medical Physiology (11th Edition). Elsevier (U.S.A). pp 936-37

Halverson, A. W, Zepplin M, and Hart E.B (1949). Relation of Iodine to the

Goitrogenic Properties of Soybeans: Four Figures. *The Journal of Nutrition* 38 (2). Pg 115.

Hamada N, Portmann L, DeGroot LJ 1 (1987).Characterization and isolation of thyroid microsomal antigen*. J Clin Invest* 79:819-825.

Hansen D, Bennedbaek FN, Hansen LK, Hoier-Madsen M, Jacobsen BB, Hegedus L. (1999). Thyroid function, Morphology and Autoimmunity in Young Patients with insulin-dependent Diabetes Mellitus.*Eur. J. Endocrinol.* 140 (6):512-8

Hathcock JN (2004). Vitamin and Mineral safety (2nd Edition).Council for Responsible Nutrition (CRN). Retrieved 25th September 2014

Hays MT (1991). Localization of human thryoxine absorption.*Thyroid* 1:241–248

Herzog V, Berndorfer U, Saber Y (1992). Isolation of insoluble secretory product from bovine thyroid: extracellular storage of thyroglobulin in covalently cross-linked form*. J Cell Biol* 118:1071-1083.

Hetzel BS. (1983). Iodine deficiency disorders (IDD) and their Eradication.

Hildebrandt JD, Scranton JR, Halmi NS (1979). Intrathyroidally generated iodide: Its measurement and origins. *Endocrinology* 105:618.

Ignarro L. J, Buga, G.M, Wood, K.S, Byrns, R.E, and Chaudhuri G (1987). Endothelium-Derived Relaxing Factor Produced and Released from Artery and Vein is Nitric Oxide. *Proc Natl Acad Sc*i USA 84: 9265- 9269..

Jing A, Wing W, Mei Y, Zhi-Min D, Yong-Chun Z, Bao-Feng Y (2005). Development of Wistar Rat Model of Insulin Resistance.*Worl.J. of Gastroenterol*. 367 9(24)

John M E. (1988), Organic Chemistry (2nd ed.), Brooks/Cole, p. 866, ISBN0534079687.

Kambe F, Seo H (1997). Thyroid-specific transcription factors.*Endocrine J*

.44:775-784.

Katagiri, R, Yuan, X, Kobayashi, S, and Sasaki S (2017). Effect Of Excess Iodine Intake on Thyroid Diseases in Different Populations: A Systematic Review and Meta-Analyses Including Observational Studies. *PLos ONE* .12(3): e0173722. Doi: 10.1371/journal.pone.0173722.

Kendall EC (1915), The isolation in crystalline form of the compound containing iodine which occurs in the thyroid: Its chemical nature and physiologic activity*. J. Am. Med. Assoc.,* 64: 2042-2043:

Kim PS, Kwon O-Y, Arvan P (1996). An endoplasmic reticulum storage disease causing congenital goiter with hypothyroidism.*J Cell Biol.* 133:517-527.

Kimura S, Kotani T, McBride OW, et al (1987). Human thyroid peroxidase: complete cDNA and protein sequence, chromosome mapping, and identification of two alternately spliced mRNAs. *Proc Natl Acad Sci.*84:5555-5559.

Ko, GT, So WY, Tong P, Ma RC, Kong AP, Ozaki R, et al. (2010). Hypoadiponectinaemia enhances waist circumference as a predictor of glucose intolerance and clustering of risk factors in Chinese men. *Diabetes Metab*.36(3):192-7. [Medline].

Kotani T, Umeki K, Matsunaga S, Kato E, Ohtaki S (1986). Detection of autoantibodies to thyroid peroxidase in autoimmune thyroid diseases by micro-ELISA and immunoblotting*. J Clin Endocrinol Metab* 62:928-933.

Kotyzováa D, Eybla V, Mihaljevičb, M, Glattre, E (2005) Effect of Long-term Administration of Arsenic(iii) and Bromine with and without Selenium and Iodine Supplementation on the Element Level in the Thyroid of Rat. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*., 149(2):329–33.

Kotyzováa D, Eybla V, Mihaljevičb, M, Glattre, E (2005) Effect of Long-term Administration of Arsenic(iii) and Bromine with and without Selenium and Iodine Supplementation on the Element Level in the Thyroid of Rat.

Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub., 149(2):329–33.

Kwon, D.Y, Daily, J.W, Kim, H.J and Park, S (2010). Antidiabetic Effects of Fermented Soybean Products on Type II Diabetes. *Nutri Res*. 30(1):1-13. doi: 10.1016/j.nutres.2009.11.004. PMID:20116654

Laurberg P, et al. (2006). The Danish investigation on iodine intake and thyroid disease. Danish Thyroid: status and perspectives. *European Journal of Endocrinology*. 155:219-220.

Laurenberg P, Pedersen KM, Hreidarsson A, Sigfusson N, Iversen E, Knudsen PR. (1998) Iodine intake and the pattern of thyroid disorders: A comparative epidemiological study of thyroid abnormalities in the elderly in Iceland and in Jutland, Denmark. *J Clin Endocrinol Metab*. 83:765-769

Lenarcie B, Krishnan G, Borukhovich R, Ruck B, Turk V, Moczydlowski E. (2000). Saxiphilin, a saxitoxin-binding protein with two thyroglobulin type1 domains, is an inhibitor of papain-like cysteine. *J Biol Chem* 275:15572 -15577.

Leonard R J (2003). Essentials of Medical Physiology (3rd Editon). Elsevier (U.S.A). pp 587-99.

Leonard R. J (2003). Essentials of Medical Physiology (3rd Editon). Elsevier (U.S.A). pp 587-99.

Levy O, Ginter CS, de la Vieja A, Levy D, Carrasco N (1998). Identification of a structural requirement for thyroid Na+/I- symporter (NIS) function from analysis of a mutation that causes human congenital hypothyroidism. *FEBS Lett.* 429:36-40.

Li CL, Chen SY, Lan C, Pan WH, Chou HC, Bai YB, et al (2010). The effects of physical activity, body mass index (BMI) and waist circumference (WC) on glucose intolerance in older people: A nationwide study from Taiwan. *Arch Gerontol Geriatr*. Mar 3 2010;[Medline].

Lindberg B, Ericsson UB, Ljung R, Ivarsson SA, (1997). High prevalence of thyroid antibodies at diagnosis of Insulin-dependent diabetes mellitus in swedish children. *J Lab. Clin. Med*. 130 (6) 585-9

Loh KC (2000). Amiodarone-induced thyroid disorders: A clinical review. *Postgrad Med J* 76:133–140.

Lu, J, Zeng, Y, Hou, W, Zhang, S, Li, L, Lou, X, Xi, W, Chen, Z and Xiang, M (2012). The Soybean Peptide Aglycin Regulates Glucose Homeostasis in TypeII Diabetic Mice via IR/IRS1 Pathway. *Journal of Nutritional Biochemistr*y. 23(11):1449. doi:10.1016/j.jnutbio.2011.09.007

Lupica S (2002). Thyroid Metabolism of Glucose.http//www.University of Liverpool.com.home-diabetes-blood glucose-glucose and metabolism-thyroid metabolism of glucose.

Macini, A, Segni, C, Raimondo, S, Olivieri, G, Silvestrini, A, Meucci, E and Curro, D (2016).Thyroid Hormones, Oxidative Stress and Inflammation.*Meidators of Inflammation.* 2016:6757154. Doi.10.1155/2016/6757154. PMCID:PMC4802023

Magnusson RP, Gestautas J, Taurog A, Rapoport B (1987). Molecular cloning of the structural gene for porcine thyroid peroxidase.*J Biol Chem.* 262:13885-13888.

Mayerhofer A, Amador AG, Beamer WG, Bartke A (1988). Ultrastructural aspects of the goiter in cog/cog mice. *J Heredity.* 79:200-203.

McCarrison, R. (1933) The goitrogenic action of soya-bean and ground-nut. *Indian Journal of Medical Research*.Vol.21 p179.

Menahan L A and Weiland, O. The Role of Thyroid Function in the Metabolism of Rat Liver with Particular Reference to Gluconeogenesis. *Eur J Biochem*. 10 188

Messina, M and Redmond, G. (2006). Effects of Soy Protein and Soybean Isoflavones on Thyroid Function in Healthy Adults and Hypothyroid Patients: A Review of the Relevant Literature. *Thyroid.* 16 (3):249-58.

Metwalley, K.A and El-Saied, A.R (2014). Glucose Homeostasis in Egyptian Children and Adolescents with B-Thalassemia major: Relationship to Oxidative Stress. *Indian Journal of Endocrinology and Metabolism*. 18(3):33-9. Doi:10.40103/2230-8210.131169.

Miller DW (2006). Extrathyroidal Benefits of Iodine.*J Ame Phys Sur*. 11(4): 106-10

Miller J.K., Swanson E.W. (1973): Metabolism of ethylenediamine

Misra, H. P. and Fridovich, I.(1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase."*J Biol Chem.* 25; 247(10):3170-5.

Moit F and Rousset B (2010). Thyroid Hormone Synthesis And Secretion. Http//Www.Thyroid Disease Manager.Com. Last Updated July 1 2010. Retrieved February 2012

Mokuno T, Uchimura K, Hayashi, R, Hajakawa, N et al (1998). Glucose Transporter 2 Concentrations In Hyper- And Hypothyroid Rat Livers. *J. of Endo* 160, 285-89

Morand S, Chaaraoui M, Kaniewski J, Deme D, Ohayon R, Noel-Hudson MS, Virion A, Dupuy C (2003). Effect of iodide on nicotinamide adenine dinucleotide phosphate oxidase activity and Duox2 protein expression in isolated porcine thyroid follicles.*Endocrinology* 144:1214-1248.

Muers HE, Dunn JT (1996). The combined action of two thyroid proteases releases T4 from the dominant hormone-forming site of thyroglobulin. *Endocrinology*. 137:3279-3285.

Mundy GR, Shapiro JL, Bandelin JG, Canalis EM, Raisz LG (1976). Direct stimulation of bone resorption by thyroid hormones.*J Clin Invest* 58:529–534.

Nakagawa H and Ohtaki S (1984). Partial purification and characterization of two thiol proteases from hog thyroid lysosomes. *Endocrinology* 115:

33-40.

Nakamura Y, Ohtaki S, Makino R, et al (1989). Superoxide anion is the initial product in the hydrogen peroxide formation catalyzed by NADPH oxidase in porcine thyroid plasma membrane. *J Biol Chem* 264:4759-4761.

Niccoli P, Fayadat L, Panneels V, et al (1997). Human Thyroperoxidase in its Alternatively Spliced Form (TPO2) is Enzymatically Inactive and Exhibits Changes in Intracellular Processing and Trafficking. *J Biol Chem* 272:29487-29492.

Nilsson R, Ehrenberg L, Fedoresak I (1987). Formation of potential antigens from radiographic contrast media. Acta Radiol 28:473–477.

Norman J (2011). Your Thyroid Gland. Endocrine Disorders –Thyroid Diseases. Retrieved November 2012

Ohguni S, Notsu K, Kato Y (1995). Correlation of Plasma Free Thyroxine Levels with Insulin Sensitivity and Metabolic Clearance Rate of Insulin in Patients with Hyperthyroid Graves' Disease. *Intern Med* 34:339-341.

Olatunbosun, S. (2011).Glucose Intolerance. Medscape Reference [On-line information]. Available online at http://emedicine.medscape.com/article/119020-overview through http://emedicine.medscape.com.Accessed December 2011.

Oppenheimer JH, Koerner D, Schwartz HL, Surks MI (1972). Specific Nuclear Triiodothyronine-Binding Sites in Rat Liver and Kidney. *J Clin Endocrinol Metab* 35:330–333

Otken A, Akcay S, Cakir M, Girisken I, Kosucu P, Deger O. (2006). Iodine Status, Thyroid Function, Thyroid Volume and Thyroid Autoimmunity in Patients with Type 1 Diabetes Mellitus in an Iodine-Replete Area.*Diabetes Metab.* 32 (4):323-9.

Panda, S, Anand, K, Patil, S (2009).Soy Sterols in the Regulation of Thyroid Functions, Glucose Homeostasis and Hepatic Lipid Peroxidation in

Mice.*Food Research International.*42 (8):1087-92.

Paneels V, Macours P, Van den Bergen H, Braekman JC, Van Sande J, Boeynaems JM (1996). Biosynthesis and metabolism of 2-iodohexadecanal in cultured dog thyroid cells*. J Biol Chem* 271:23006 -23014.

Park, S, Kim, D.S, Kim, J.H, Kim, J.S and Kim, H.J. (2012). Glyceollin-Containing Fermented Soybeans Improved Glucose Homeostasis in Diabetic Mice. *Nutrition*. 28(2):204.

Postiglione MP, Parlato R, Rodriguez-Mallon A, Rosica A, Mithbaokar P, Maresca M, Marians RC, Davies TF, Zannini MS, de Felice M, di Lauro R (2002). Role of the Thyroid-Stimulating Hormone Receptor Signaling in Development and Differentiation of the Thyroid Gland.*Proc Natl Acad Sci* . 99:15642-7.

Rabelo R, Schifman A, Rubio A, Sheng X, Silva JE (1995). Delineation of thyroid hormone-responsive sequences within a critical enhancer in the uncoupling protein gene.*Endocrinology* 136:1003–1013.

Rabie A, Favre C, Clavel MC, Legrand J(1977). Effects of thyroid dysfunction on the development of the rat cerebellum, with special reference to cell death within the internal granular layer.*Brain Res*120:521–531.

Ravindra M, Vivek R J, Ayaz K M, Manjunath G, Jeevan K S, Prakash M , Revathi P S, Shivaraj B (2011). A comparative Study on Iodination of Normal and Diabetic Serum*. Int. J. Appl. Biol.and Pharm. Tech*. 2: 1. ISSN 0976-4550

Rawitch AB, Pollock G, Yang SX, Taurog A (1990). The location and nature of the N-linked oligosaccharide units in porcine thyroid peroxidase: studies on the tryptic glycopeptides. In: Thyroperoxidase and Thyroid Autoimmunity. Eds: Carayon P, Ruf J. John Libbey Eurotext, London, pp 69-76.

Rhoades R.A, Tanner, GA (2004). Medical Physiology (2nd Edition).Lippincott

Williams and Wilkins.Pp 597-602, 625,630. ISBN: 0781719364

Roubsanthisuk W, Watanakejorn P, Tunlakit M, Sriussadaporn S. (2006). Hyperthyroidism induces glucose intolerance by lowering both insulin secretion and peripheral insulin sensitivity. *J Med Assoc Thai.*89 : S133-S140.

Ruf J, Czarnocka B, de Micco C, Dutoit C, Ferrand M, Carayon P (1987). Thyroid peroxidase is the organ-specific 'microsomal' autoantigen involved in thyroid autoimmunity. *Acta Endocrinol Suppl* (Copenh) 281:49-56.

Salau B.A, Ketiku AO, Ajani EO, Ezima EN, Idowu GO and Soladoye MO (2008).iodine contents of some selected roots/tubers, cereals and legumes consumed in Nigeria*. Afr.JBiotech.*7(23),pp.4328 4330. Available online at http://www.academicjournals.org/AJB. ISSN 1684–5315 © 2008 Academic Journals

Sang Z, et al. (2012).Exploration of the safe upper level of iodine intake in Chinese adults; a randomized double blind trial.*American Journal of Clinical Nutrition.* 95:371.

Sharpless,G. H., J. Pearsons and G. S. Prato (1939) Production of goiter in rats with raw and with treated soybean flour. J. Nutrition, 17: 545.

Shepard TH, Pyne GE, Kirschvink JF, McLean M. (1960). Soybean Goiter: Report of three cases. *N Engl J Med.* 262:1099–1103.

Sinaiko, A. R, Caprio, S (2012). Insulin Resistance. *J. Pediatr*. 161 (1): pg 11

Singh AK, Jiang Y, White T and Spassova D (1997). Validation of Non-radioactive Chemiluminescent Immunoassay Methods For The Analysis Of Thyroxine And Cortisol In Blood Samples Obtained From Dogs, Cats And Horses*. J. Vet. Diagn. Invest*. 9: 261-68

Sirkarwar, M.S, Patil, M.B (2010). Antidiabetic Activity of *Crateva nurvala* Stem Bark Extracts in Alloxan-induced Diabetic Rats. 2 (1). Pg 19

Soleimani M, Greeley T, Petrovic S, Wang Z, Amlal H, Kopp P, Burnham CE (2001). Pendrin: an apical Cl(-)/OH(-)/HCO(3)(-) exchanger in the

kidney cortex. *Am J Physiol Renal Physiol* 280:356-364.

Song Y, Massart, C., Chico-Galdo, V., Jin, L., De, M., V, Decoster, C., Dumont, J. E. & Van Sande, J. (2010). Species Specific Thyroid Signal Transduction: Conserved Physiology, Divergent Mechanisms. *Mol. Cell Endocrinol.* 319: 56-62.

Spiro MJ (1977). Presence of a Glucuronic acid-containing Carbohydrate Unit in Human Thyroglobulin.*J Biol Chem* 252:5424.

Sterling K, Lazarus JH, Milch PO, Sakurada T, Brenner MA (1978). Mitochondrial thyroid hormone receptor: localization and physiological significance*. Science* 201:1126–1129.

Sudipta, C, Srikanta, G, Ipsita, S, and Madhusudan D (2007). Thyroid Dysfunction Modulates Glucoregulatory Mechanism In Rat. *Indian J. Exper.Bio.* 45, 545-53

Swai, A.B.M, Mclarty, D.G, Mtinangi, B.L, Tatala, S, Kitange, H.M, Mlingi, N, Rosling, H, Howlett, W.P, Brubaker, G.R (1992). Diabetes is Not Caused by Cassava Toxicity: A study in Tanzanian Community. *The American Diabetes Association*. 15(10):1378-85.doi: 10.2337/diacare

Targovnik HM, Vono J, Billerbeck AEC, et al (1995). A 138-nucleotide deletion in the thyroglobulin ribonucleic acid messenger in a congenital goiter with defective thyroglobulin synthesis.*J Clin Endocrinol Metab* 80:3356 -3360.

Thyroid Foundation of Canada (2012)

Uyttersprot N, Pelgrims N, Carrasco N, et al (1997). Moderate doses of iodide in vivo inhibit cell proliferation and the expression of thyroperoxidase and Na+/I- symporter mRNAs in dog thyroid. *Mol Cell Endocrinol.* 131:195-203.

Vanderpum, M. P. J (2018). Epidemiology of Thyroid Diseases. Encyclopedia of Endocrine Diseases. 2nd Edition. Pp

Varshney, R. and Kale, R. K. (1990): “Effects of Calmodulin Antagonists on Radiation-induced Lipid Peroxidation in Microsomes.” *Int’l j Rad. Biol.* 58:733-743

Venturi S, Donati F M, Venturi A, Venturi M (2000). "Environmental iodine deficiency: A challenge to the evolution of terrestrial life?".Thyroid :*official journal of the American Thyroid Association*10 (8): 727–9. doi:10.1089/10507250050137851. PMID11014322.

Verma, S, Pradeep, Kumar G., Malini, L, Nivsarkar, M, Apurna, S (1991). Superoxide Dismutase Activation in Thyroid and Suppression in Adrenal Novel Pituitary Regulatory Routes. 282 (2). P310-11

Vitti P, Rago T, Aghini-Lombardi F, Pinchera A (2001). Iodine deficiency disorder among the Hungarian Community.*Pub.Health Nutr*. 4: 529535

Walters, E, Mclean P (1967). Effect Of Thyroidectomy On Pathyways Of Glucose Metabolism In Lactating Rat Mammary Gland. *Biochem, J*. 105 (2): 615-23

Wetter T.J, Gazdag, A.C, Dean, D.J, Cartee, G. D (1999). Effect of Calorie Restriction on In-Vivo Glucose Metabolism by Individual Tissues in Rats. *Am J Physiol*. 276(4): E728-38. doi: 10.1152/ajpendo. 1999.276.4.E728.PMID: 10198310.ld

WHO (2001). Assessment of the Iodine Deficiency Disorders and Monitoring their Eliminatioin. Geneva: world health organization, WHO document/NHD/01.1

Wild S, Roglic G, Green A, Sicree R, King H (May 2004). "Global prevalence of diabetes: estimates for 2000 and projections for 2030". *Diabetes Care* 27 (5): 1047–53. doi:10.2337/diacare.27.5.1047. PMID15111519.

Wilgus, H. F. X. Gassner,a. B. Patton and B. G. Gustavson (1941). The goitrogenieity of soybeans. J. Nutrition, 22: 43.

Wolff (1969). Iodide Goiter and the Pharmacological Effect of Excess Iodide. *The American Journal of Medical Electronics.* 47. 101-2

Wolff J, Chaikoff IL (1948).“Plasma inorganic iodide as a homeostatic regulator of thyroid function”.*J. Bio Chem* 174 (2): 555-64

World Health Organisation (1999). Diabetes Criteria- Interpretation Of Oral Glucose Tolerance Test.

Wynn JO (1961). Components of the serum protein-bound iodine following administration of I131-labeled hog thyroglobulin. *J Clin Endocrinol Metab* 21:1572.

Yalçin B, Ozan H (2006). "Detailed investigation of the relationship between the inferior laryngeal nerve including laryngeal branches and ligament of Berry".*J. of the Ame. Coll. of Surgeons* 202 (2): 291–6. doi:10.1016/j.jamcollsurg.2005.09.025. PMID16427555.

Yen PM (*2001*).Physiological and molecular basis of thyroid hormone action*. Physiol Rev* 81*,* 1097 –1142*.*

Yessoufou, A, Ategbo, A. G, Girard, A, Prost, J, Dramane, Moutairou, A. H, Khan, N.A (2006). Cassava-enriched diet is not diabetogenic rather it aggravates diabetes in rats. 20 (6). 579-8