# 1. LITERATURE REVIEW

# 1. INTRODUCTION

Type 2 Diabetes is a chronic, slowly progressive disease with an inherited and /or environmental component. Individuals may have a genetic predisposition to this disease and then particular environmental factors, such as a virus, dietary factors or lifestyle, or chronic inflammatory conditions, results in the onset of the disease. Type 2 Diabetes results in a decrease in the production of insulin by the pancreas, and/or insulin resistance, where the insulin released is not effective in lowering the blood glucose concentration due to it not allowing the transportation of glucose into the cells of various tissues throughout the body. In either case there is the resultant increase in blood glucose concentration leading to damage of many body systems, especially the blood vessels and nerves, resulting in retinopathy, nephropathy, neuropathy and heart disease. Type 2 Diabetes can also increase the risk of miscarriage and congenital malformations to almost twice that is seen in normal pregnancies (Hampton, 2004).

The prevalence of Type 2 Diabetes is increasing rapidly worldwide, primarily due to the increase of sedentary lifestyles and obesity (Uusitupa, 2002), with an estimated 177 million people suffering from it worldwide in 2002 (Dunstan *et. al.,* 2002; World Health Organization, 2002). This figure is expected to reach at least 324 million by the year 2025, which is approximately

5.5% of the adult population (Meisinger *et. al.,* 2006a). This increase will occur mainly in the developing countries, (170%), due to their aging population, the more sedentary lifestyle and poor diet as they prosper, which also leads to obesity, compared to developed counties who are expecting an increase of 42% (Dunstan *et. al.,* 2002; Saudek, 2002). Most people who have the disease in the developed world are $\geq$ 65 years of age while those in the developing countries are between the ages of 45 and 65 years (Bonow & Gheorghiade, 2004). Having Type 2 Diabetes can shorten life expectancy by as much as fifteen (15) years (Gillies *et. al.,* 2007). Type 2 Diabetes was also once thought of as a disease of the aged; however it is now being diagnosed in young adults, adolescents and even children. In the USA between 30-50% of new cases of Type 2 Diabetes are diagnosed in youth between the ages of 9 and 19, compared to 2% twenty years ago (Novick, 2001). In 2002 it was estimated that at least 987,000 deaths (1.7% total mortality) occurred from Type 2 Diabetes or its complications, and is the fifth leading cause of death world wide (Roglic *et. al.,* 2005). Many of those people suffering from diabetes are also unaware of it and hence are not receiving treatment, leading to more complications, with the proportion of undiagnosed diabetes being as high or even higher then 50% (Roglic *et. al.,* 2005). Impaired glucose tolerance also leads to an increased mortality rate of approximately 40%, independent of whether they progress to diabetes or not and hence the impact of hyperglycaemia and insulin resistance is larger than that associated with diabetes alone (Roglic *et. al.,* 2005).

In England there are approximately 1.3 million people who have been diagnosed with Type 2 Diabetes, and approximately 5% of the total National Health Services resources and up to 10% of hospital inpatient resources are used for the treatment of people with diabetes (Gillies *et. al.,* 2007). Prevention of the disorder and maintenance of people diagnosed with Type 2 Diabetes would certainly lower the costs of this disease to the national budget of England and all countries. In the United States of America it is estimated that the cost per diabetic patient is between $4,000 and $5,000 per annum, with costs increasing as the severity of the disease progresses (Nichols and Brown, 2005). The national debt is US$92 billion annually in direct costs and an additional US$40 billion in indirect costs such as time off work etc (Petersen and Shulman, 2006). In Germany the direct costs for the treatment of patients with diabetes is 14.2% of the total health care costs, with approximately 26.5 million or one third of the population suffering from the disease (Koster *et. al.,* 2006).

In Australia there are 854,325 individuals that have been diagnosed with Type 2 Diabetes and registered with the National Diabetes Register, being 3.97% of the total population (Deed, 2009), and it is estimated that another 400,000 who are not registered, have either a pre-diabetic or do not realise that they have diabetes, this being one of the highest recorded prevalences in the developed world. The percentage of the population has risen to 3.6% of the population in the year 2004-2005 (Barit and Cooper, 2009) and is continuing to rise rapidly. It has also been suggested that the average Australian has about a

one in fourteen chance of developing Type 2 Diabetes during their lifetime, and if impaired fasting glucose and impaired glucose tolerance are included, then approximately one in four Australians would be classified as having a glucose uptake disorder (Dunstan *et. al.,* 2002) . The Australian Aboriginal population have an incidence of Type 2 Diabetes of 9.9% , while that of an older white population based in the Blue Mountains of New South Wales was 9.3%t, and if those with an impaired fasting glucose are added it is 15.8% (Cugati, *et. al.,* 2007), suggesting that it is not just an aboriginal problem in Australia.

The magnitude of the problem is enormous with billions of dollars being spent annually by most western nations on the treatment of this disease and its complications. In developed countries Type 2 Diabetes claims about 9% of the National Health Budget (World Health Organisation, 2002) and in many countries it may account for 10% or more of the total health budget (Roglic *et. al.,* 2005). Diabetes is Australia's sixth leading cause of death from disease and seventh overall, including accidents (Australian Bureau Statistics, 2000). This leads to a huge economic burden on the country. Days lost from work due to the disease are numerous which a major economic burden is also. A study in Australia has shown that people with diabetes cost approximately 38% more ($1017) than non-diabetics in the hospital system (Clarke *et. al.,* 2006) and this is an increasing burden on the society as a whole, financially, as well as with beds being taken from other medical needs.

Today there are several medications used to ameliorate the effects of diabetes and its complications by controlling blood glucose levels (BGL). These include oral hyperglycaemic drugs such as the sulfonylureas, biguanides and thiazolidinediones, and insulin which is used in the end stages of Type 2 Diabetes. Although the effects of the hypoglycaemic drugs appear to be sufficient and BGL are normalised initially, they still tend not to stop the micro-vascular effects of the disease (Moss *et. al.,* 1994) and their effectiveness appears to diminish overtime, therefore, an increased amount of a drug, or combination of drugs, must be taken to have the same effect (Cook *et. al.,* 2007).

Since people with impaired glucose tolerance have a high risk of developing Type 2 Diabetes, they need to be diagnosed and preventative measures put into place as soon as possible. This can be achieved through regular BGL tests and also looking at other risk factors such as family history, waist-to-hip circumference ratios and exercise levels and then a change in lifestyle factors such as diet and exercise should be initiated early, but may also include some pharmacological agents. Thus, the development of new and improved medications for prevention of Type 2 Diabetes will also assist in the real cost of this disease**,** as well as raising the well being, of those predisposed to the disease.

The recorded use of plants in the treatment of diabetes dates back to approximately 1550 BCE (Gray & Flatt, 1997b), and in recent years many

people have again turnrd to Natural Therapies as a treatment alternative. More than 400 traditional herbal remedies used in the treatment of diabetes by various cultures have been recorded; however only a few of these have received medical and scientific evaluation to determine effectiveness, efficacy, side effects and toxicity (Baily and Day, 1989). The World Health Organisation has recommended that medical and scientific examinations of such plants be undertaken (World Health Organisation Expert Committee on Diabetes Mellitus, 1980).

## 1.1 GLUCOSE HOMEOSTASIS

### 1.1.1Glucose Regulation

Glucose is the major energy source for all mammalian cells; however the BGL must be maintained within a very narrow concentration range of 4.5 – 5.0 nml/L in order to avoid its own toxic effects. This level is maintained even with the variable intake of sugars and use of energy, and the homeostatic control is achieved by the matching of the flux of glucose into and out of the plasma through tightly regulated secretion of insulin and glucagon from the pancreatic islet cells with hyperglycaemia itself acting as a β-cell toxin and bringing about apoptosis of the β–islet cells resulting in decreased insulin secretion (Chang *et. al.,* 1996).

The homeostatic process begins in the gastrointestinal tract where the uptake of glucose into the plasma is regulated by transport across the gastrointestinal endothelium. The homeostatic process is also controlled by the liver as it regulates BGL by glucose uptake and storage along with production of glucose in times of need. Other tissues, in particular muscles, which are involved in the uptake and storage of the glucose, are also extremely important in maintaining the balance. The balance of uptake and storage is precisely matched by the output of glucose from glycogenolysis, and gluconeogenesis. After glucose intake this balance is upset, and the return to homeostasis is dependent on three processes that occur simultaneously: (1) Insulin secretion by the β-cells, (2) Glucose uptake by the liver, muscle where it is stored as glycogen, and adipose tissues (Pilkis & Granner, 1992) or metabolised for energy, and (3) gluconeogenesis and glycogenolysis are suppressed through insulin-induced inhibition of glucagon secretion and blockage of the actions of glucagon on the liver.

### 1.1.2 Glucose Transport

Skeletal muscle expresses relatively high levels of the glucose transporter GLUT4, which is responsible for insulin-stimulated glucose transport (Rea & James, 1997) and low levels of GLUT1, which is responsible for basal noninsulin-dependent transport (Zorzano *et. al.,* 1996). During fasting states, GLUT4 is localized mainly in intracellular vesicles, and it is translocated to cell

membrane in response to insulin. Once transported into the cell, glucose is rapidly phosphorylated by hexokinase (HK) to glucose-6-phosphate (G6Pase) and this keeps the concentration of intracellular glucose low, ensuring the continual movement of glucose across the plasma membrane (Postic *et. al.,* 1994; Mandarino *et. al.*, 1995). There are several different hexokinases with HKI being expressed in all tissues and HKII in insulin sensitive tissues, (Postic *et. al.,* 1994) with GLUT4 and HKII being involved in insulin mediated glucose utilization, and GLUT1 and HK1 being involved in basal glucose uptake.

Insulin resistance, where the released insulin is less effective, is a predominant feature of Type 2 Diabetes and is often an indication of a prediabetic state. Since glucose transportation is one of the first rate limiting steps in glucose metabolism and is often down regulated in both skeletal muscle and adipose tissue in patients with Type 2 Diabetes (Zierath *et. al.,* 1994; Zierath, 1995), it is suggested that this may play a major role in the development of impaired glucose uptake. Zierath and colleagues (1996) have shown that insulin resistance is not due to a deficiency of GLUT4, which is still translocated to plasma membrane by insulin, however, the defect is likely to be due to an altered translocation of GLUT4 or in the altered fusion of the GLUT4 vesicles to the plasma membrane. Intracellular Free Fatty Acids (FFA) block the insulin mediated activation of GLUT 4 and its translocation, as well as decreases in intracellular glucose levels possibly due to the interference of the insulin

signalling pathway with the ability of insulin to activate phosphatidylinositol-3-kinase (PL3K) being inhibited (Bloomgarden, 2006).

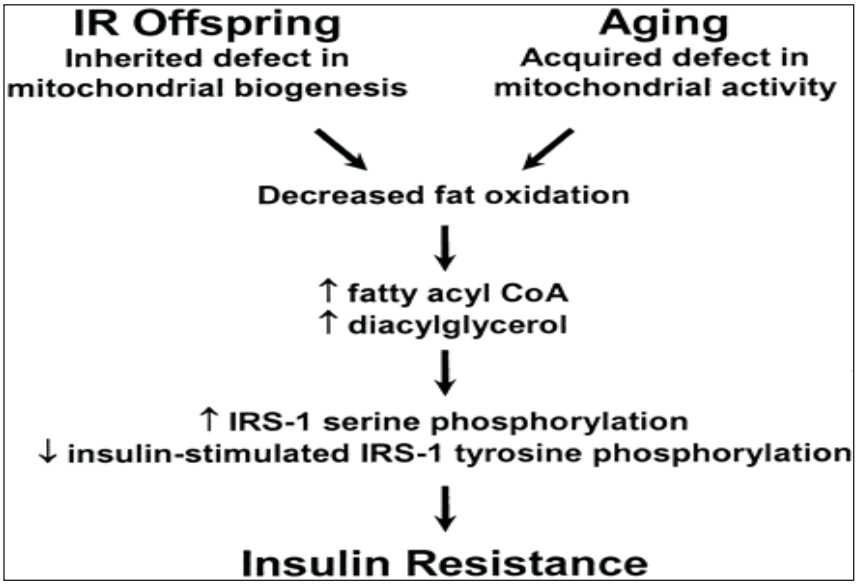



Figure 1.1: Schematic depicting the central role of the mitochondria and impaired lipid oxidation in causing insulin resistance in offspring of type 2 diabetic patients (IR offspring) and the elderly (Befroy *et. al.,* 2007).

The membrane protein PC-1 contains two sites, a phosphodiasterase site that does not appear to be related to insulin sensitivity, and a somatomedin B domain that binds to the insulin receptor α subunit which prevents insulin-induced conformational change and by preventing insulin receptor signalling through the inhibition of tyrosine kinase activity. The PC-1 content shows an inverse relationship with insulin sensitivity and therefore may play a role in insulin resistance (Bloomgarden, 2006).

Signalling through the PI3K pathway is crucial for metabolic responses to insulin (Alessi & Downes, 1998). The binding of insulin to its receptor activates the insulin receptor tyrosine kinase, which initiates a cascade of signalling events. The initial step is the tyrosine phosphorylation of insulin receptor substrate 1 (IRS1), which then binds to PI3K, which activates its catalytic unit. The tyrosine phosphorylation of IRS1 is the first signalling step that has been shown to have reduced sensitivity in the diabetic patient (Danielsson *et. al.,* 2005). The enzyme AMP-activated protein kinase (AMPK), which is stimulated by an increase of AMP/ATP, plays a role in several cellular and metabolic processes during exercise, including the increase of skeletal muscle fatty acid oxidation, and glucose transport (Sriwijitkamol *et. al.,* 2007). The lipid products of PI3K activate protein kinase B (Akt), which in turn mediates many of the metabolic activities of insulin (Kohn *et. al.,* 1996; Martin *et. al.,* 1996) and may be involved in the events leading to translocation of GLUT 4 to the cell membrane resulting in the uptake of glucose into the cell (Cortright & Dohm, 1997). Phosphorylation of AS160, a substrate of Akt, is required for the translocation of GLUT4 and may be impaired in insulin resistance (Hakan *et. al.,* 2005).

### 1.1.3 The Gastrointestinal Tract

Glucose homeostasis begins in the stomach and small intestine, where acute changes in BGL affect the gastric motor functions. Gastric emptying is slower

during hyperglycaemia and accelerated during hypoglycaemia (Horowitz *et. al.*, 2002), thus absorption of glucose into the portal system from the small intestine, is affected by the rate of gastric emptying, this being controlled by feedback to the satiety centre of the hypothalamus via the hormone ghrelin, which also has a direct effect on the pancreas promoting β-cell regeneration (Irako *et. al.,* 2006). A diet high in natural fibre will slow gastric emptying and hence delay the absorption of glucose from the small intestine (Riccardi *et. al.*, 2003).

The classical model of glucose absorption across the apical membrane of the enterocyte in the lumen of the small intestine is via the Na+/glucose co-transporter (SGLT1) and exits into the portal system across the basolateral membrane via the facilitative transporter GLUT2 (Reviewed in Kellett and Brot-Laroche, 2005). Fructose is transported by the specific facilitative transporter, GLUT5 (Burant *et. al.,* 1992), while GLUT2 transports both fructose and glucose (Cheeseman, 1993). Initially it was thought that GLUT2 was only found at the basolaterial membrane: however, it has now been located in the apical membrane as well (Corpe *et. al.,* 1996).

The glucose transported by SGLT1 promotes insertion of GLUT2 into the apical membrane within minutes of high glucose levels reaching the lumen of the small intestine resulting in a much faster and greater absorption of glucose (Helliwell & Kellett, 2002). The α-glucosidase inhibitors such as acarbose reduce the breakdown of complex carbohydrates in the gut and possibly

decrease the diet induced up-regulation of transport of sugars across the gut wall (Casirola & Ferraris, 2006).

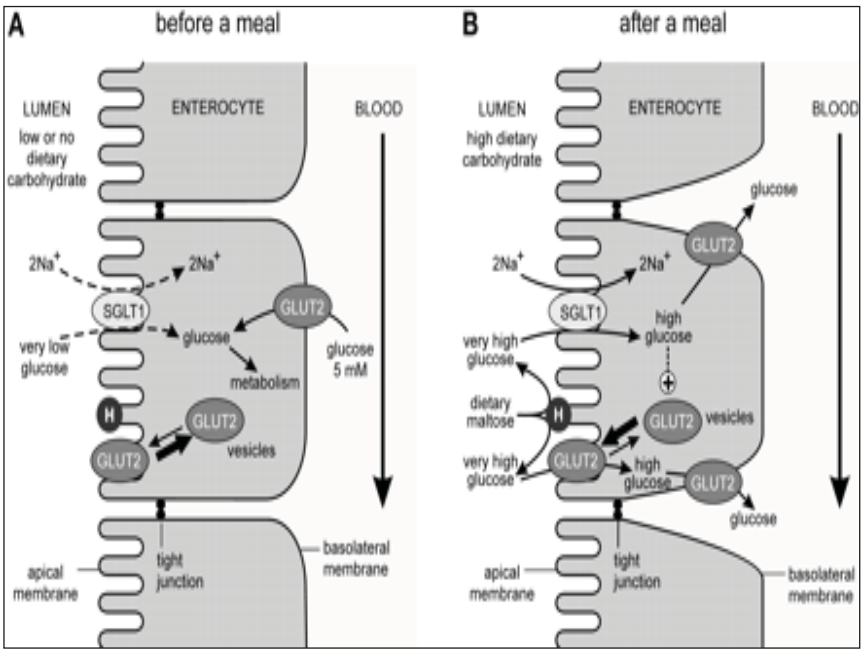



Figure 1.2: The apical GLUT2 model of intestinal glucose absorption before (*A*) and after (*B*) a meal. (Reviewed in Kellett and Brot-Laroche, 2005).

### 1.1.3.1 Glucagon-like-peptide-1 and Gastric Inhibitory Polypeptide

Glucagon-like-peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) are hormones, produced in the L cells and are released in the stomach postprandial and enhance glucose-stimulated insulin secretion (Ahren, 1998; Drucker, 1998), while GLP-1 also inhibits glucagon secretion, delays gastric emptying and stimulates insulin biosynthesis (Ahren, 1998; Drucker, 1998) and

GIP has been shown to increase glucagon secretion in the healthy individual (Meier *et. al.,* 2003).

GLP-1 is rapidly degraded by the enzyme dipeptidyl peptidase IV in the stomach and has only very short-term effects, therefore it is not suitable for treatment of Type 2 Diabetes; however, new analogues have been produced that have the same effect but a much longer half life and inhibitors of dipeptidy peptidase IV that decrease the GLP-1 degradation making them feasible in treatment protocols (Holst 2006). GLP-1 and Exendin-4 (an analogue of GLP-1) have been shown to increase β-cell mass in adult rodents (Stoffers *et. al.,* 2000) and Tourrel and colleagues (2001) demonstrated that GLP-1 or Exendin-4 applied during the neonatal period would exert both a long and a short-term beneficial effect on β-cell mass and glucose homeostasis in mice, though the mechanism has not yet been elucidated. The use of GLP-1 or Exendin-4 in the pre-diabetic period delays the onset and limits the severity of Type 2 Diabetes, because of its β-cell replenishing effect in diabetic mice (Tourrel *et. al.*, 2002), by possibly increasing β-cell mass allowing the pancreas to continue to produce insulin for an extended period of time or an increased amount of insulin. Lee and colleagues (2007) have shown that GLP-1 and Exendin-4 improves insulin sensitivity by restoration of insulin signalling and also decreases hepatic gluconeogenesis in diabetic ob/ob mice and the use of inhibitors of GLP-1 breakdown and analogues may be useful in the treatment of Type 2 Diabetes

(Todd and Bloom, 2007); however, the long-term effects of these need to be studied further.

### 1.1.4 The Pancreas

#### 1.1.4.1 Insulin

Insulin is synthesized in the endoplasmic reticulum of the β-islets and transported to the Golgi complex, where the insulin-containing secretory granules are formed through budding, stored until needed and finally secreted by exocytosis upon stimulation (Reviewed in Wollheim *et. al.,* 1996). Insulin secretion is highly regulated and responds to very small changes in the BGL. This occurs by various glucose-derived signals and the potentiating and inhibitory effects of various endocrine and paracrine influences of other nutrients (Bock *et. al.,* 2007), hormones and neurotransmitters. The potentiating and inhibitory effects appear to remain intact during the pre-diabetic state and after the onset of Type 2 Diabetes (Bock *et. al.,* 2007), however, abnormal secretion of insulin is seen regularly due to a primary insulin secretion defect, exhaustion of the pancreas, glucose toxicity or a combination of the above (Porte 1991).

Insulin secretion, by the β-cell, is coupled to glucose metabolism, with a major signal being the closure of $K^+$ATP (potassium adenosine triphosphate)-dependent channels in the cell membrane due to the increased cytosolic ATP

with a glucose induced increase in the cytoplasmic ATP/ADP ratio. This ratio serves as the major regulator of the $K^+$ATP-dependent channels in the cell membrane, with the closure of these channels depolarising the cell membrane. This in turn leads to the opening of the voltage-dependent calcium ($Ca^{2+}$) channels, raising the intracellular $Ca^{2+}$ concentration, which results in insulin secretion (Koster *et. al.,* 2005). Any alteration to the metabolic signalling, sensitivity of the $K^+$ATP -dependent channels to metabolites or the number of active channels could disrupt the signalling process and hence alter insulin secretion. Congenital hyperinsulinism, a rare recessive disorder, which results in constitutive insulin secretion irrespective of blood glucose levels and a decrease in K + ATP-dependent channels expression, has been implicated in this disorder. Conversely, mutations that result in an increased expression of the $K^+$ATP-dependent channels activity should decrease the glucose sensing by the β-cell and hence decrease insulin secretion leading to higher blood glucose levels (Koster *et. al.,* 2005).

Secondary messengers, such as GLP-1 and GIP and neurotransmitters which increase glucose sensitivity, also play an important role in insulin secretion (Howell *et. al.,* 1984). The insulin producing β-islets cells are richly innervated by the autonomic nervous system (Porte *et. al.*, 1990), and the parasympathetic stimulation increases insulin secretion by the binding of acetylcholine to muscarinic receptors through the activation of adenylate

cyclase-cyclic adenosine monophosphate (cAMP), GIP and GLP-1 and phospholipase C. Cyclic AMP modulates the influx of $Ca^{2+}$ through voltage-gated $Ca^{2+}$ channels and potentiates $Ca^{2+}$-dependent exocytotic events which act independently of the effect on $Ca^{2+}$ channels (Ammala *et. al.,* 1993), and is also associated with the conversion of insulin from proinsulin, the prohormone of insulin (Ahmad *et. al.,* 1991b). Proinsulin is converted in the β-cell to insulin and C-peptide by a series of proteolytic steps and are both stored in the secretory granules, and released along with some partially processed and intact proinsulin (McFarlane, 1991).

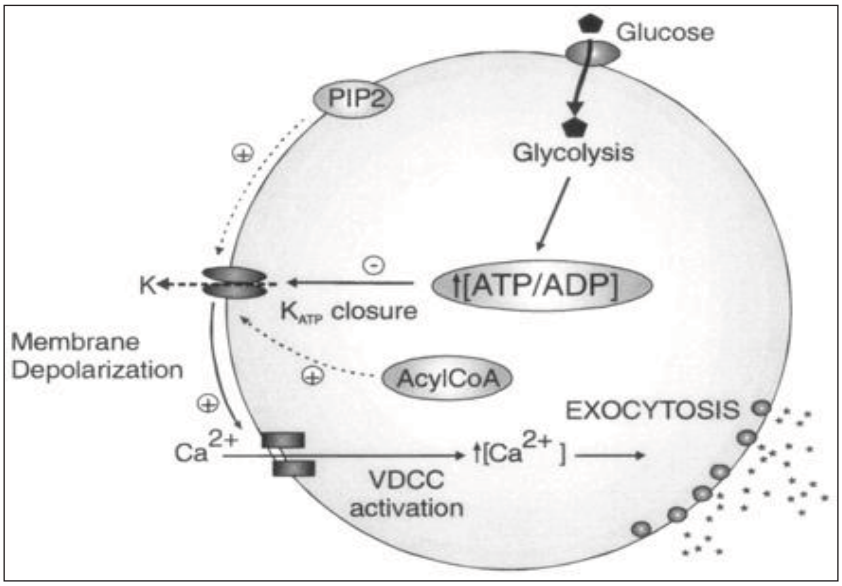



Figure 1.3: The role of pancreatic $K_{ATP}$ channel in insulin secretion. Elevated blood glucose increases glucose metabolism in the ß-cell and elevates $[ATP]/[ADP]_i$. This metabolic signal closes $K_{ATP}$ channels, causing depolarization, activation of voltage-dependent $Ca^{2+}$ channels, $Ca^{2+}$ entry, and insulin exocytosis. Various additional effectors, including $PIP_2$ and acyl CoAs, act to modulate ATP sensitivity of the channel and can thereby affect the coupling of metabolism to secretion (Koster *et. al.,* 2005).

An increase in proinsulin is a feature of Type 2 Diabetes. Steiner and James (1992) have suggested that regulation in the conversion of proinsulin to insulin may be defective in some diabetic patients resulting in high circulating proinsulin levels. Over-stimulation of β-cells increases the proinsulin to insulin ratio (Bjorklund & Grill, 1999) and FFA also increases the ratio. This may be due to the delay in processing of the prohormone (Furukawa *et. al.*, 1999), resulting in a lesser hypoglycaemic effect on muscle, adipose tissues and the liver than that of insulin. At least two prohormone convertases (PC3/PC1 and PC/2) are involved in the cleavage of proinsulin into insulin, whose activity is regulated by an acidic gradient between the Golgi and secretory granules and by $Ca^{2+}$ ions of the cells (Lindberg *et. al.,* 1991).

Insulin is released in pulses (Goodner *et. al.*, 1977); however, this pattern is often defective in the diabetic patient (Lang *et. al.*, 1981). The β-cells are arranged in clusters within the Islets of Langerhans, which are scattered throughout the pancreas, and therefore require co-ordination of the intrinsic, pulsatile secretory activities of the cells in order to generate oscillations. Normal pulsitility is important as it could render insulin more energy-efficient and more efficient in maintaining glucose homeostasis (Bratusch-Marrain *et. al.,* 1986); however, in Type 2 Diabetes this regulation disappears, and may lead to insulin receptor down-regulation and glucose intolerance (Lang *et. al.,* 1981). Variations in metabolism (Gilon *et. al.,* 1993), cytoplasmic $Ca^{2+}$ concentrations

(Gylfe, *et. al.,* 1991), the intrinsic nervous system (Lang *et. al.,* 1981) and hormones such as GLP-1 (Bratusch-Marrain *et. al.,* 1986), neural signalling (Daniel & Henderson, 1967) and β-cell insulin receptor expression (Xu & Rothenberg, 1998) have all been implicated in the regulation of plasma insulin oscillations. However, in some Type 2 Diabetic patients the insulin is still released in pulses indicating that the impaired secretory response to glucose may be related to impaired metabolism (Lin *et. al.,* 2002).

Insulin secretion is biphasic with phase one being the release of stored insulin and phase two the synthesis and release of more insulin in response to a continued high BGL. The first phase of insulin release is low or absent in the Type 2 Diabetic patient, while the second phase is present but may be decreased (Rang *et. al.,* 2000;) and the first phase release is also absent or impaired in patients who are in a prediabetic state (Beard *et. al.,* 1987). A homeostatic state is established by the release of insulin when the BGL rises and the release of glucagon as BGL falls with their secretion controlled by nutritional (eg glucose and saturated fat intake), hormonal (eg Adrenalin) and neural (eg sympathetic nervous system) signals (Liang & Matschinsky, 1994). There are many secretagogues such as certain amino acids, fatty acids, acetylcholine and GLP-1, glucose which can initiate insulin secretion (Bosch *et. al.,* 1998); however, there are many other secretagogues that will only modulate the secretion of insulin. Insulin resistance increases the demands on insulin secretion and hence the β-

cells. This in turn leads to the increase in synthesis of insulin and β-cell replication and neogenesis. However, any long term increased demands for insulin secretion can negatively affect β-cells by over-stimulation, resulting in their eventual apoptosis and hence a decrease in insulin production.

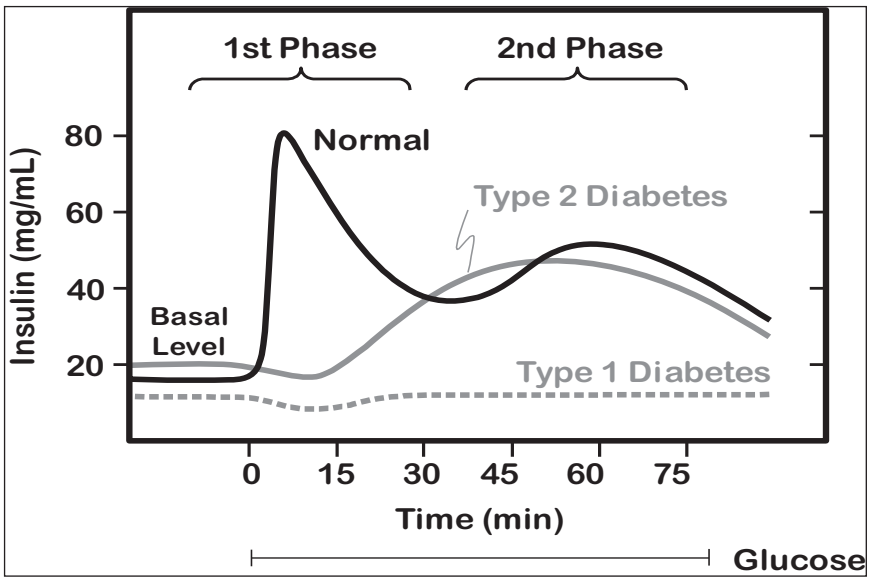



Figure 1.4: Insulin Secretion in normal, Type 1 and Type 2 Diabetic states. (Rang *et. al.,* 2000)

Amylin, a pancreatic polypeptide stored and secreted along with, and with the same apparent stimuli as insulin (Kahn *et. al.,* 1998), currently does not have any reported physiological role but has been noted for its absent expression in Type1 Diabetics, which is expected. Amylin deficiency so far has not been reported to be associated with any pathophysiological conditions or symptoms (Gebre-Medhin *et. al.,* 1998); however, amylin replacement therapy helps to regulate gastric-emptying, produce the feeling of satiety and therefore decreases food intake, and suppresses the abnormal glucagon release with meals which

may give an indication of its role in the pathogenesis of Type 2 Diabetes (Owen, 2006).

It has been suggested that as the amylin fibres refold to a β-conformation and oligomerises to form insoluble fibrils, and then accumulate in the cells where there is a decline in β-cell function (Westermark, 1994). This folding may be inhibited by intragranular heterodimer formation with insulin but not proinsulin; however, if there is irregular processing of the proinsulin then the inhibition breaks down and the folding occurs. The ineffective processing of pro-islet amyloid polypeptide may also result in the incorrect folding of the polypeptide and/or the inability to bind to insulin and hence the deposition of amyloid (Clark *et. al.,* 1987). The percentage of amylin fibres may determine the degree of hyperglycaemia in Type 2 Diabetes (Westermark, 1994). In Type 2 Diabetes, insulin secretion decreases with the progression of the disease (UK Prospective Diabetes Study Group, 1998), although this decrease is not explained by autoimmune dysfunction or chronological age (Clausson *et. al.,* 1994). The amylin to insulin ratio increases with age (Edwards *et. al.,* 1996) which may bring about the decrease in insulin secretion that occurs with age. This, in turn, may be due to toxicity of amyloid resulting in cell death or loss of function due to adherence of fibrils to cell membranes interfering with cycling of membrane proteins and hence secretions (Botto *et. al.,* 1997).

Calcium, an intracellular second messenger, is the critical trigger for insulin exocytosis from β-cells (Wollheim & Sharp, 1981; Wollheim *et. al.,* 1996). Plasma membrane depolarisation is brought about by the generation of ATP and other metabolic coupling factors, which promotes $Ca^{2+}$ influx into the cell, with the rise in intracellular $Ca^{2+}$ concentration then triggering the exocytosis of insulin. The hyperpolarization of the mitochondrial membrane glucose also increases the $Ca^{2+}$ concentration, activating $Ca^{2+}$ sensitive dehydrogenases in the Krebs cycle producing glutamate, which also aids in the calcium-stimulated exocytosis (Wollheim *et. al.,* 1996).

### 1.1.4.2 Glucagon

Glucagon, a hormone produced by the alpha-cells of the pancreas (Unger & Orci, 1981) and also in small amounts in the L-cells of the intestine and stomach, acts on the liver when the BGL is low to stimulate glucose production. Kimball and Murlin, (1924); Grimelius and colleagues (1976), and Orci and colleagues, (1983) all showed that glucagon works by mobilizing glucose reserves in the liver through glycogenolysis and gluconeogenesis, preventing hypoglycaemia as well as stimulating lipolysis and ketogenesis in adipose tissue providing FFAs for the liver (McGarry, 1979; Carlson *et. al.,* 1993).

The regulation of the alpha cell is complex, with a range of hormonal, nutrient and neural stimuli all involved in its function, glucose being the most

significant, as it profoundly suppresses glucagon secretion in the normal cell. There are a number of amino acids which will also suppress glucagon secretion, with arginine being the most effective in humans (Larsson & Ahren, 2000); however this effect varies from species to species. Insulin acts directly on glucagon secretion via a local endocrine effect (Stagner & Samols, 1992) while somatostatin acts by a paracrine effect (Taborsky, 1983).

In Type 2 Diabetes the abnormalities seen in alpha cell function appear to be a result of impaired glucose sensing or alpha cell resistance, with an increase in gluconeogenesis being a major outcome. In the Type 2 Diabetic patient the alpha cell fails to down-regulate its secretion of glucagon in response to hyperglycaemia as a result of the impaired glucose sensing (Gastaldelli *et. al.,* 2000).

Miniglucagon, the COOH-terminal fragment processed from glucagon, is a potent and efficient inhibitor of insulin secretion and impairment of its metabolism could lead to impaired insulin secretion. The mechanism of action is thought to be mediated by a pertusses toxin-sensitive G protein linked to a pathway that involves the potassium channel opening and the resulting membrane repolarisation (Dalle *et. al.,* 1999).

### 1.1.4.3. Somatostatin

The D cells of the Islets of Langerhans and the hypothalamus secrete somatostatin, which is known as the growth hormone release-inhibiting factor and provides a local inhibitory regulatory action on insulin within the islets (Rang *et. al.,* 2000).

### 1.1.5. The Liver

The liver plays a major role in maintaining glucose homeostasis. In the fasting state, the liver, through glycogenolysis, breaks down glycogen to form glucose, and gluconeogenesis (the synthesis of glucose from substances such as various amino acids, lactate and pyruvate), releases glucose into the blood to maintain BGL within the very narrow concentration range of around 5mM (DeFronzo, 1997). This process is critical to maintain a glucose supply for the neural tissues, which cannot use other forms of energy. However, uncontrolled gluconeogenesis results in excessive hepatic glucose production, which is a major factor in high BGLs (DeFronzo, 1997). Upon glucose absorption into the portal system, insulin is released and carried to the liver, where it binds with specific receptors on the hepatocyte. The importance of this can be seen by the fact that it contains approximately 50% of the insulin binding receptors of the body, and suppresses the hepatic glucose output and induces glucose uptake, via the glucose transporters GLUT 2 and GLUT 1 in the liver (DeFronzo, 1997).

Gluconeogenesis is a cAMP/protein kinase A (PKA) – dependent process initiated by glucagon and possibly some of the inflammatory cytokines. This process is generally inhibited by insulin in a number of places including the decrease in glucagon secretion from the alpha cells in the pancreas, blocking the glucagon signalling pathway by activating a cAMP phosphodiesterase. There is also suppression of key gluconeogenic genes such as phosphoenolpyruvate carboxykinase (PEPCK), 6Pase and p38 which plays a stimulatory role in hepatic gluconeogenesis (Cao *et. al.,* 2005) results in decreased glucose production. Insulin secretion may be decreased in Type 2 Diabetes and therefore these mechanisms are not inhibited, resulting in a continual increase in glucose output by the liver resulting in high fasting blood glucose levels (Cao *et. al.,* 2005).

There is an increasing interest in the role of the liver in the pathogenesis of Type 2 Diabetes. Liver fat content has been shown to correlate with several features of insulin resistance in both normal and overweight individuals. This is independent of Body Mass Index (BMI) (Body mass/Height$^2$), and intra-abdominal or overall obesity, with a liver enzyme, alanine aminotransferase (ALT) being used to measure liver fat content and this has been shown to predict the incident of Type 2 Diabetes (Sattar *et. al.,* 2007). Wannemethee and colleagues (2005) have shown that both ALT and γ-glutamyltransferase (GGT) is independent predictor's of Type 2 diabetes; however their role in diabetes has

not yet been determined. Elevated ALT and GGT however are still considered within the "normal" range were predictive of Type 2 Diabetes independent of obesity and alcohol intake in older men; however, results also indicate that ALT maybe ethnically based as the results varied in Japanese and Korean men. Wannemethee and colleagues (2005) suggested that levels of ALT and GGT correlated with increases of hepatic fat and this may lead to hepatic insulin resistance; however, when adjusted for insulin resistance there was still a 3-4 fold increase in the development of Type 2 Diabetes. They went on to suggest that the correlation of ALT and GGT with tumor necrosis factor - α (TNF-α) and Interleukin-6 (IL-6) may play a role; however, adjustments for this still did not account for the increased risk. Oxidative stress was put forward as another possibility; however, further studies need to be carried out to determine if this is the mechanism.

### 1.1.6. Skeletal Muscle

Skeletal muscle composes approximately 40% of body mass and accounts for approximately 75% of the uptake of glucose after glucose absorption which is converted to glycogen (DeFronzo, 1988) and hence plays a major role in the maintenance of a normal blood glucose level.

Physical exercise increases insulin-mediated glucose utilization, reflecting an adaptation in muscle brought about by a local contraction-mediated

mechanism. GLUT4 is also stimulated by long-term, low-frequency stimulation of muscle (Etgen *et. al.,* 1993). Exercise does not alter the receptor function or the number of receptors but does increase the amount of GLUT4 present within the cells. This can be seen after a single training session (Dela *et. al.,* 1993; Ren *et. al.,* 1994), along with bringing about the translocation of GLUT4 to the plasma membrane (Zorzano *et. al.,* 1996).

Insulin resistance has been linked to inactivity and obesity due to the disuse of muscles, which, in itself, can lead to obesity and brings about a decrease in GLUT4 expression; however, GLUT1 expression is enhanced (Castello *et. al.,* 1993). Intracellular FFA also block the insulin mediated activation of GLUT 4 and its translocation, as well as decreases in intracellular glucose levels possibly due to the interference of the insulin signalling pathway with the ability of insulin to activate PI3K being inhibited (Bloomgarden, 2006). Insulin resistance within the muscle tissue has also been linked to dysregulation of fatty acid metabolism, due to a genetic defect in the mitochondrial oxidative-phosphorylation process and is seen as a predictor of insulin resistance in relatives of Type 2 Diabetic patients (Befroy *et. al.,* 2007).

The membrane protein PC-1 contains two sites, a phosphodiasterase site that does not appear to be related to insulin sensitivity, and a somatomedin B domain that binds to the insulin receptor $\alpha$ subunit which prevents insulin-induced conformational change and by preventing insulin receptor signalling

through the inhibition of tyrosine kinase activity. The PC-1 content shows an inverse relationship with insulin sensitivity and therefore may play a role in insulin resistance (Bloomgarden, 2006).

Signalling through the PI3K pathway is crucial for metabolic responses to insulin (Alessi & Downes, 1998). The binding of insulin to its receptor activates the insulin receptor tyrosine kinase, which initiates a cascade of signalling events. The initial step is the tyrosine phosphorylation of IRS1, which then binds to PI3K, which activates its catalytic unit. The tyrosine phosphorylation of IRS1 is the first signalling step that has been shown to have reduced sensitivity in the diabetic patient (Danielsson *et. al.,* 2005). The enzyme AMPK, which is stimulated by an increase of AMP/ATP, plays a role in several cellular and metabolic processes during exercise, including the increase of skeletal muscle fatty acid oxidation, and glucose transport (Sriwijitkamol *et. al.,* 2007). The amount of AMPK within the myocyte is increased with exercise and this leads to increased mitochondrial biogenesis and function. This, in turn, leads to increased muscle glucose disposal, and fatty acid oxidation (Sriwijitkamol *et. al.,* 2007). The lipid products of PI3K activate Akt, which mediates many of the metabolic activities of insulin (Kohn *et. al.,* 1996; Martin *et. al.,* 1996) and may be involved in the events leading to translocation of GLUT 4 to the cell membrane resulting in the uptake of glucose into the cell (Cortright & Dohm, 1997). Phosphorylation of AS160 is required for the

translocation of GLUT4 and may be impaired in insulin resistance (Hakan *et. al.,* 2005). Bloomgarden (2006) suggests that an increase in intracellular FFA will prevent this translocation from occurring; especially considering that the improvement in insulin resistance brought on by exercise could be due to decreases in the amount of intracellular triglycerides. PTEN is a lipid/protein phosphatase that can negatively regulate this pathway (Butler *et. al.,* 2002) and possibly be a factor in the pathogenesis of Type 2 Diabetes. Bandyopadhyay and colleagues (2005) showed that increased expression of the PI-3 adaptor subunits p85/55/50 decreased PI3K activity and hence, attenuated insulin sensitivity in skeletal muscle.

Muscle contraction increases insulin sensitivity, but also stimulates glucose uptake independent of insulin. The contraction signalling pathway is distinct from the insulin pathway because the effects of insulin and contractions on glucose uptake are additive and contraction does not increase insulin receptor kinase or PI3K activity (Cortright and Dohn, 1997). Cortright and Dohn (1997) suggest that both the contraction and insulin signalling pathways can be blocked by calcium channel blockers indicating that the pathways may converge; however, there are two distinct GLUT4 pools which are being targeted by the different pathways.

A relationship between the fatty acid composition of skeletal muscle membrane phospholipid and insulin resistance has been demonstrated (Borkman

*et. al.,* 1993; Vessby *et. al.,* 1994), as the greater the percentage of polyunsaturated fatty acids the better the insulin reaction. Pan and colleagues (1995) showed a relationship between ‘5 desaturase activity and insulin resistance, although the mechanism has not been elucidated. Intramyocellular lipid accumulation has also been associated with whole-body insulin resistance and defective insulin signalling (Viramaki *et. al.,* 2001); however this is independent of body weight and physical fitness.

The majority of glucose that enters skeletal muscle cells in response to inulin is converted to glycogen. In Type 2 Diabetic patients, and in those who are insulin resistant, glycogen synthesis is severely impaired (DeFronzo, 1997). Glycogen synthase (GS) is the key insulin-regulated enzyme controlling the rate of glycogen synthesis (Dent *et. al.,* 1990; Lawrence & Roach, 1997) and if deficient or ineffective the rate of glycogen synthesis decreases leading to increased glucose levels.

GLUT5 plays a major role in skeletal muscle uptake of fructose, although the role of this transporter is not clear at this stage. It has been noted that its expression is high in diabetic muscle and that Pioglitazone, one of the Thiazolidinedione (TZD) medications, will decrease the overexpression of GLUT5 and hence decrease blood glucose levels (Stuart *et. al.,* 2007).

### 1.1.7. Adipose Tissue

Adipose tissue is more than just an energy storage organ, but is a secretory organ which produces a variety of proteins that influence the metabolism of the body. There are many hormones and cytokines produced by adipocytes that effect food intake, carbohydrate and lipid metabolism and energy expenditure, including resistin, TNF-α, Il-6, plasminogen activator inhibitor 1, angiotensin II, adiponectin, Acylation stimulating protein (ASP) and Leptin. Each of these factors interact with each other to increase or decrease body weight, energy expenditure and insulin resistance.

#### 1.1.7.1. Resistin

Resistin has been reported to contribute to insulin resistance (Berger, 2001; Flier, 2001; Steppen *et. al.,* 2001a; Steppen *et. al.,* 2001b) and it is suggested that it may be a key factor linking obesity with insulin resistance, as its expression and circulating levels are increased in obese, insulin resistant mice and inhibited by insulin-sensitizing peroxisome proliferators-activated receptors (PPAR's) (Steppen *et. al.,* 2001a). However, other groups have found that there is a fall in resitin in obese and insulin resistant animals (Rajala *et. al.,* 2003). Resistin levels are elevated in both obesity and genetic models of insulin resistance (Steppen *et. al.,* 2001a), with its expression specific to white adipose tissue. It acts by suppressing insulin's ability to stimulate glucose uptake into

adipose cells (Steppen *et. al.,* 2001a). It has been suggested that the mechanism of the actions of resistin is mediated by signals to the PPAR-γ receptor in insulin-responsive tissues to modulate the insulin-signalling pathway (Steppan *et. al.,* 2001a). In adipocyte tissue cultures, glucose transport was reduced in response to insulin in the presence of resistin, while anti-resistin produces the opposite effect (Kim *et. al.,* 2001). In resitin gene knockout mice a decreased fasting glucose, improved glucose tolerance and enhanced insulin sensitivity was observed possibly due to activation of AMPK and reduced gluconeogenesis enzyme production (Banerjee *et. al.,* 2004).

### 1.1.7.2. Leptin

Differentiated adipocytes in white adipose tissue, is the major producer of leptin with the plasma concentration and mRNA expression being directly related to the severity of obesity (Ahima and Flier, 2000). Insulin and leptin act within the central nervous system to activate thermogenesis and inhibit food intake (Havel, 2000). Both hormones act as critical signals for the long term regulation of energy homeostasis and body adiposity. They do this in part by activating short-term signals of satiety, possibly through a common signalling pathway – PI3K (Havel, 2001; Niswender *et. al.,* 2001). Leptin also appears to have significant effects on hepatic insulin action and peripheral glucose utilization, possibly mediated through the central nervous system (Barzilai *et. al.,* 1999; Chinookoswong *et. al.,* 1999). It has been suggested that changes in

insulin and glucose levels are what mediates the effects of energy intake on leptin production by adipocytes (Havel, 2001). Leptin appears to be able to control TNF-α production and macrophage activation (Loffreda *et. al.,* 1998) as well as TNF-α and IL-6 stimulating adipocyte leptin production (Abdel-Hafez *et. al.,* 2002; Lau *et. al.,* 2002). Leptin also improves insulin sensitivity through activation of AMPK, which in turn controls malonyl-CoA concentrations within the cell, thereby inhibiting acetyl-CoA carboxylase (Minokoshi *et. al.,* 2002), leading to a decrease in lipogenesis. Conversely, the leptin-signalling pathway inhibits insulin signalling and may lead to insulin resistance (Howard and Flier, 2006).

The structure of leptin is similar to that of other cytokines, along with its receptor-induced signalling pathways leading to the suggestion that it might also play a role as a pro-inflammatory factor (Otero *et. al.,* 2005) and chronic inflammation has been linked to insulin resistance.

### 1.1.7.3. Adiponectin

Adiponectin, a large molecular weight plasma protein, that has at least three isoforms (low-molecular weight, medium-molecular weight and high-molecular weight complexes) which are produced and secreted by adipose tissue, and have been shown to enhance fatty acid oxidation in muscles, which modulates lipid and glucose metabolism. Plasma adiponectin levels are inversely correlated

with the severity of insulin resistance and are now considered to be a major link between obesity and insulin resistance, with a decrease in the high-molecular weight isoform and in the high-molecular weight isform-to-total adiponectin being more highly correlated with glucose intolerance (Nakashima *et. al.,* 2006; Snijder *et. al.,* 2006). As insulin sensitising agents such as TZD's increase adiponection levels (in particular the high-molecular weight form (Nakashima *et. al.,* 2006)) in humans (Lindsay *et. al.,* 2002), support is given to this theory. Administration of adionpectin has improved insulin sensitivity in mice models, while the complete reversal of insulin resistance in lipoatrophic animals required co-administration of leptin (Yamauchi *et. al.,* 2001).

The mechanism of action of adiponectin has not as yet been elucidated however, available data suggests that it reduces hepatic glucose production, increases hepatic insulin sensitivity, and increases muscle glucose utilization. This may occur by increasing fat oxidation, reducing hepatic fatty acid synthesis and thereby reducing circulating free fatty acid levels and intramyocellular lipid accumulation (Snijder *et. al.,* 2006; Qi *et. al.,* 2006). Yamauchi and colleagues (2002) have suggested that adiponectin increases insulin sensitivity through activation of AMPK (with the high molecular weight adiponectin being the most insulin sensitising), and Kadowaki and Yamauchi (2005) show that adiponectin decreases mRNA expression of phosphoenolpyuravate carboxykinase and G6Pase, both of which are essential enzymes in gluconeogenesis. Adiponectin

appears to also reduce the inflammatory response of TNF-α; however, adiponectin production in humans is reduced by TNF-α and IL-6 (Bruun *et. al.,* 2003).

Yokoyama and colleagues (2006) have suggested that adiponectin is associated with nonoxidative glucose disposal, which is reduced in the Type 2 Diabetic patient and controls insulin sensitivity via glycogen synthesis. Hojlund and colleagues (2006) suggested that insulin sensitivity is improved by enabling the switching from lipid to glucose oxidation within the muscle cell and with excess glucose being stored as glycogen, whereas in the diabetic subject low adiponectin leads to impaired insulin activation of glycogen synthase. It also appears to have major anti-inflammatory properties that may play a role in decreasing insulin resistance and diabetes (Chandran *et. al.,* 2003) and Winer and colleagues (2006) have shown a relationship between adiponectin levels and the inflammatory marker, C Reactive Protein (CRP), hence, there may be a link between low grade inflammation and diabetes. In a study on db/db mice by Todoric and colleagues (2006), it was noted that when fed a high saturated fat diet the adiponectin levels were reduced by down regulation of the adiponectin gene. However, those fed with a low fat diet had a higher level of adiponectin gene expression, and the inclusion of n-3 series marine polyunsaturated fatty acids to the diet reversed the down-regulation, indicating a possible role in FFA metabolism.

### 1.1.7.4. Tumour Necrosis Factor

Tumour Necrosis Factor α is an inflammatory cytokine that when over-expressed in adipose tissue, as often found in obesity, interferes with insulin receptor signalling, and is a possible cause of the development of insulin resistance in obesity (Hotamisligil *et. al.,* 1993). It is also over-expressed in muscle tissue, in the obese individual (Kern *et. al.,* 1995), possibly bringing about insulin resistance. Tumour Necrosis Factor α decreases adiponectin secretion and increases the production of Free Fatty Acids (FFA), increasing the size of the adipocyte leading to insulin resistance (Bloomgarden, 2006). Tumour Necrosis Factor α inhibits the phosphorylation of IRS1 in response to insulin, suppressing insulin action and downstream signalling as well as inhibiting adiponection expression. Several serine/threonine kinases that are activated by TNF-α contribute to this inhibition of insulin signalling, including c-Jun $NH_2$-terminal kinase (JNK), inhibitor of nuclear factor-κ B Kinase (IKK) and protein kinase C-τ. JNK has also been implicated in islet cell inflammation and death leading to β-cell dysfunction and defective insulin production. Tumour Necrosis Factor α and other inflammatory mediators have been implicated in other insulin resistance cascades including the activation of inducible nitric oxide synthase, production of reactive oxygen species, regulation of suppressor of cytokine signalling proteins and alterations in AMP-K and mTOR pathways in obesity (Emanuelli *et. al.,* 2001; Perreault & Marette,

2001; Furukawa *et. al.,* 2004; *Lin et. al.,* 2004; Khamzina *et. al.,* 2005). Tumour Necrosis Factor α down regulates the expression of electron transport genes in visceral and subcutaneous adipose tissue and in the Type 2 Diabetic patient. This down-regulation is independent of obesity and appears to be specific for adipose tissue (Dahlman *et. al.,* 2006).

### 1.1.7.5. Visfatin

Visfatin or pre-B cell colony-enhancing factor is a cytokine that is expressed in visceral adipose tissue and works synergistically with IL-7 to promote β-cell precursors as well as increasing adipocyte differentiation. It has also been noted that it is secreted by activated lymphocytes, monocytes and neutrophils, and hence, is believed to play a role in innate immunity, though the mechanism is unknown at this stage (Chen *et. al.,* 2006). The blood levels of visfatin correlate with obesity and also with a high fat diet and Chen and colleagues (2006) suggest that this might be important in its action. Intravenous injection of visfatin would lead to an acute fall in glucose levels in normal mice, independent of insulin and also in mice chronically infected with an adeno virus encoding visfatin resulting in high levels of the hormone and significantly lower blood glucose and insulin levels.

Chen and colleagues (2006) have shown that Visfatin exhibits an insulin mimetic effect in stimulating glucose uptake by muscle and adipose tissue and in

inhibiting gluconeogenesis. Visfatin binds to and activates insulin receptors, resulting in phosphorylation and activation of downstream signalling molecules, though there is no competition between insulin and Visfatin for the insulin receptor, suggesting that they are recognised by different parts of the receptor. Visfatin also mimics insulin in the insulin transduction pathway, as it induces tyrosine phosphorylation of insulin receptors IRS-1 and -2, and activation of PI3K, Akt and MAP kinase (Fukuhara *et. al.,* 2005). Interestingly, serum visfatin levels increase with progressive β-cell deterioration in Type 2 Diabetic patients unlike insulin (Lopez-Bermejo *et. al.,* 2006) and may aid in glucose uptake when insulin secretion is low due to insulin mimicking effects.

### 1.1.7.6. Other Adipokines

The interaction of complement factors C3, B and D, factor B and adipsin results in the formation of an ASP within adipocytes, with its main functions being to increase the efficiency of triacylglycerol synthesis, to stimulate glucose uptake, to activate diacylglycerol acyltransferase, and inhibit the activity of hormone sensitive lipase within the adipocyte (Cianflone *et. al.,* 1999). ASP promotes the storage of energy as fat whereas interfering with ASP production attenuates lipid storage and leads to obesity resistance and improves insulin sensitivity (Havel, 2002).

Retinol binding protein 4 (RBP4) is secreted by adipose tissue and prevents extraction of retinol by the kidney's and is also closely associated with GLUT4 mRNA levels as well as insulin sensitivity (Gavi *et. al.,* 2007). RBP4 levels have also been shown to correlate with waist-to-hip ratios, however, not to the percent of body fat, suggesting a role in abdominal obesity (Gavi *et. al.,* 2007) which is a predisposing factor for Type 2 Diabetes. Ribel-Madsen and colleagues (2009) suggest that the elevated plasma RBP4 as seen in Type 2 Diabetes is a predominantly nongenetic phenomenon and plays only a secondary and minor role in the development of insulin resistance.

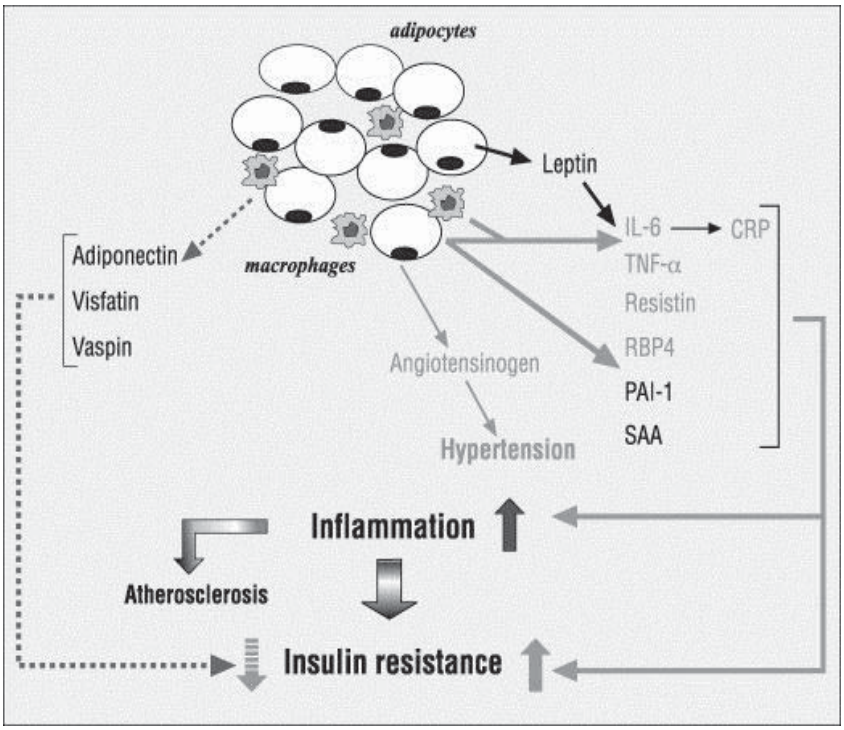



Figure 1.5: Adipose Tissue, adipokines and Insulin Resistance. (Antuna-Puente *et. al.,* 2008)

Serum amyloid A is expressed in adipose tissue and is significantly increased in obesity and Type 2 Diabetes, however, its role in the disease is

unknown. It is an acute-phase inflammatory protein and often used as a marker for coronary events (Leinonen *et. al.,* 2003).

Vaspin is a serine protease-inhibitor strongly expressed by visceral adipose tissue and is stimulated in mouse (Hida *et. al.,* 2005) and human (Kloting *et. al.,* 2006) obesity. Insulin or insulin sensitizing drugs will normalise its expression (Curat *et. al.,* 2006) and if Vaspin is injected into an animal with obesity due to a high-fat-diet, insulin resistance is improved (Hida *et. al.,* 2005).

### 1.1.8. Glucose Uptake

There are three main molecular characteristics of β-cell glucose metabolism that are of importance in the maintenance of homeostasis: (1) The expression of the high capacity, low affinity glucose transporters GLUT 2 in both the β-cells, and hepatocytes (Thorens *et. al.,* 1988; Johnson *et. al.,* 1990), which allows glucose to equilibrate across the plasma membrane, (2) Glucose phosphorylation to glucose-6-phosphate which is catalysed by glucokinase (GK), which is the rate-determining step for glycolysis (Reviewed in Matschinsky, 1996), and (3) Glycolysis produces pyruvate, which in turn is channelled to the mitochondria. This in turn generates ATP and other factors, which promote insulin secretion (Schuit *et. al.,* 1997).

Glucokinase (GK), a member of the hexokinase family, and a phosphorylating enzyme is a crucial component in the control of glucose

metabolism in the β-cells and in the hepatocytes (Printz *et. al.,* 1993b). The GK gene has two distinct promoters specific for the liver and β-cells; the hepatic promoter is regulated by insulin, whereas the β-cell promoter is constitutively active. Both GLUT2 and GK have a high $K_m$ (Michaelis constant) for glucose ensuring that glucose uptake is proportional to extracellular glucose concentrations. In Type 2 Diabetes the GK gene expression and GK activity are very low, which leads to deficient glucose metabolism in both the β-cells and the liver (Printz *et. al.,* 1993b). Fructose activates hepatic GK and when administered to the diabetic patient will partially correct the regulation of glucose production suggesting that the impaired GK activity substantially increases glucose production (Hawkins *et. al.,* 2002).

Decreased concentrations of GLUT2 or a decreased expression of GK (Bali *et. al.,* 1995; Grupe *et. al.,* 1995; Terauchi *et. al.,* 1995) alters the signal given by high glucose concentrations that regulates insulin secretions, which leads to a decrease in insulin release.

## 1.2. TYPE 2 DIABETES

Type 2 Diabetes is the most common of all the metabolic disorders and it is characterised by insulin resistance (a decrease in the response to insulin by peripheral tissues) and β-cell dysfunction (inadequate insulin release) (DeFronzo, 1988), and β-cell apoptosis (Butler *et. al.,* 2003). There is also

commonly found an increase in endogenous glucose production and decrease peripheral glucose uptake (Bogardus *et. al.,* 1984; DeFronzo, 1988), along with the ability of insulin to promote glycogen synthesis and storage being decreased (Shulman *et. al.,* 1990; Thorburn *et. al.,* 1990). In 1976 and 1977, Unger and Orci suggested that diabetes is a bihormonal abnormality: (1) glucagon excess resulting in the overproduction of glucose and (2) the lack of insulin resulting in under utilization of glucose.

In populations with a high incidence of Type 2 Diabetes (Prima Indians, the highest reported incidence in the world; Mexican-Americans and Pacific Islands, Aboriginal Australians and Torres Strait Islanders and Asian Indians) (Zimmet *et. al.,* 1984; Ramachandran *et. al.,* 1997; Harris *et. al.,* 1998; Dunstan *et. al.,* 2002) insulin resistance occurs early in life and precedes glucose intolerance. The β-cell will release more insulin to compensate for the lack of sensitivity, however, the β-cells reach a point of exhaustion where they can no longer secrete enough insulin to compensate for the decreased insulin sensitivity, and fasting hyperglycaemia and diabetes develops (Kahn & Porte, 1997). Poor insulin responsiveness to glucose results not only from β-cell insensitivity, but also from the metabolic derangement of diabetes, that forms a vicious cycle (Kosaka *et. al.*, 1980). The tissue of initiation and the exact process is not yet known, however there are four major candidates; β-cells, liver, skeletal muscle and adipose tissue. Petersen and Shulman (2006) suggest that the origin is the

skeletal muscle, where any of the glycogen synthesis pathway steps may be disrupted and most likely those of insulin-stimulated glucose transport and phosphorylation. However, Kahn (2008) suggests that β-cell dysfunction and number or volume of functioning β-cells appears low in those at risk of diabetes and so may play a role in the initiation of this disease.

Exogenous glucose appears in the circulation at the same rate in individuals with and without Type 2 Diabetes after ingestion of sugars (McMahon *et. al.,* 1989a; McMahon *et. al.,* 1989b; Mitrakou *et. al.,* 1992). In normal subjects the various signals such as hyperinsulinaemia, hyperglycaemia, and neural signals, act together to inhibit endogenous glucose production (Reviewed in Cherrington *et. al.,* 1987), however, in Type 2 Diabetic individuals this inhibition is suppressed and endogenous glucose production continues, which is the major cause of postprandial hyperglycaemia (Mitrakou *et. al.,* 1992). Mitochondria in skeletal muscle tend to be small and less in number resulting in reduced oxidative activity and the level of ATP synthase β which is an essential protein for respiration. In adipocytes there has been shown a decrease in size and number of mitochondria leading to a lack of ATP which in turn leads to lipid biosynthesis, and dysfunction in fatty acid oxidation and respiration (Choo *et. al.,* 2006).

There is generally a regular turnover of β-cells; however, when there is an increase in glucose there is a greater increase in β–cell apoptosis and

regeneration is less, leading to a decreased islet mass within the pancrease. β-cell mass increases during times of demand such as obesity; however a β-cell mass decrease has been implicated as one of the factors leading to diabetes (Kahn, 2008). The long term adaptation of the β-cell to differing conditions may be initiated by hyperglycaemic excursions, which elicit β-cell production

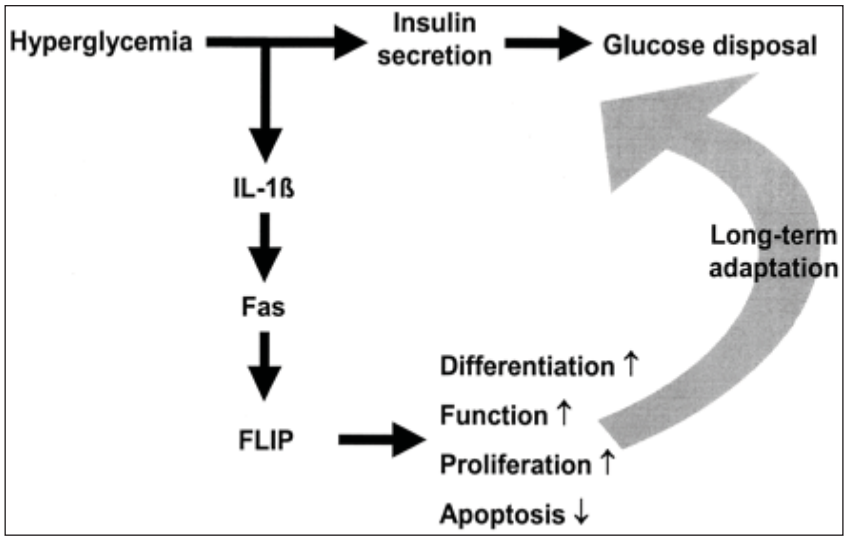



Figure 1.6: Hypothetical model illustrating the consequence of hyperglycemias on ß-cell production of IL-1ß in parallel with insulin secretion. The paracrine effect of IL-1ß induces Fas engagement, which in the presence of FLIP leads to ß-cell proliferation, differentiation, and increased function. (Donath *et. al.,* 2005).

of interleukin-1β (Maedler *et. al.,* 2002), followed by FAS up-regulation (Laybutt *et. al.,*2003), a decrease in FLICE inhibitory protein which leads to apoptosis of the cells (Maedler *et. al.,* 2002). Endoplasmic stress (ES) due to hyperglycaemia and increased lipids may be responsible for some of the β-cell apoptosis as the β-cell has a highly developed endoplasmic reticulum (ER) and

conditions that disrupt the ER functions of folding, exporting and processing of insulin leads to the accumulation of misfolded proteins. This in turn results in an adaptive process which comprises translational attenuation reducing synthesis of insulin and accumulation of improperly folded proteins, upregulation of the genes involved in the chaperone proteins to increase folding of the insulin, proteosomal degradation of misfolded proteins and apoptosis of the cell if the stress continues (Laybut *et. al.,* 2007).

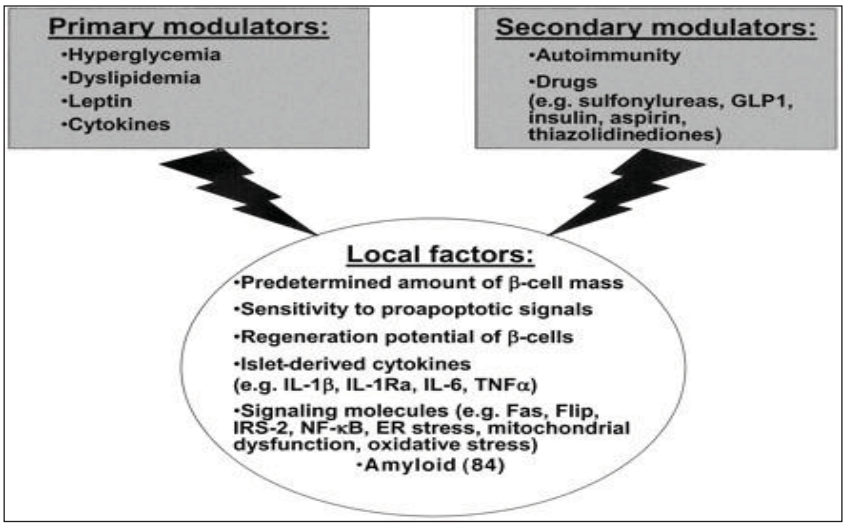



Figure 1.7: Proposed model for mechanisms regulating ß-cell mass in type 2 diabetes. Before onset of diabetes, insulin resistance may lead to transient postprandial hyperglycaemic excursions. Other factors modulating ß-cell mass may include dyslipidemia, leptin, and cytokines. Genetic predisposition to diabetes may include a predetermined amount of ß-cell mass, as well as differences in the susceptibility to apoptotic signals and in the regenerative potential of the ß-cell. Additionally, induction of local inflammatory mediators and cell death may activate the acquired immune system. Finally, drugs may protect or harm the ß-cell. GLP1, glucagon-like peptide 1; IRS-2, insulin receptor substrate 2; TNF$\alpha$, tumor necrosis factor-$\alpha$. (Donath *et. al.,* 2005).

### 1.2.1 Carbohydrates

Diabetes has long been viewed as a disorder of carbohydrate metabolism due to its hallmark feature of hyperglycaemia, which is the cause of the associated symptoms such as polyuria, polyphagia and polydipsia (Beiswenger, 2000), and also the long term complications such as nephropathy, neuropathy, and blindness (UK Prospective Diabetes Study Group, (UKPDS), 1998). The component of the diet that has the greatest influence on blood glucose is carbohydrates, while proteins and fats have a less major role, both being able to slow the absorption of carbohydrates, delaying the peak glycemic response to a mixed meal (Nuttall and Gannon, 1991), proteins will also augment insulin release when ingested in a mixed meal, thereby increasing the clearance of glucose from the blood (van Loon *et. al.,* 2000).

The World Health Organisation now recommends that dietary carbohydrates be classified according to their glycemic index (FAO/WHO, 1997), which is the measure of the change in blood glucose following ingestion of carbohydrate-containing foods. The glycemic index is the increase in blood glucose (over the fasting level) that is observed in the 2 hours following ingestion of a set amount of carbohydrate in the item and then this value is then compared with the response to a reference food (usually glucose) containing an equivalent amount of carbohydrate (Jenkins *et. al.,* 1981). Some foods (potatoes, pumpkin) result in a marked rise followed by a more or less rapid fall

in blood glucose, while other foods (green vegetables) produce a smaller peak with a more gradual decline in plasma glucose (Institute of Medicine of the National Academies, 2002). The quantity and type of carbohydrate found in different foods also influence the postprandial glucose level (Franz *et. al.,* 2002; Institute of Medicine of the National Academies, 2002) with the specific type of carbohydrate (starch or sucrose for example) not always predicting its effect on blood glucose (Wolever *et. al.,* 1994; Foster-Powell and Miller, 1995). The glycemic index of food is important to understand the effect of that food on blood glucose levels, however, the amount of that food consumed is also important, for this reason Salmeron and colleagues (1997a) have suggested the use of the glycemic load. The glycemic load can be defined as the product of the glycemic index and the amount of the carbohydrate in the serving and by summing the glycemic load contributed by individual foods the overall glycemic load of a meal can be calculated (Salmeron *et. al.,* 1997b).

Epidemiological studies form the basis for the hypothesis that a diet with a high glycemic load or glycemic index can lead to Type 2 Diabetes (Salmeron *et. al.,*1997a; Hu *et. al.,* 2001), however, one study by Salmeron and colleagues (1997b) showed that both glycemic index or glycemic load were associated with the risk of Type 2 Diabetes, except when adjusted for cereal fibre intake, and Meyer and colleagues (2000) showed no correlation between glycemic index or glycemic load and the development of Type 2 Diabetes. Liu and colleagues

(2000) showed that substituting foods with a low glycemic index for those with a high glycemic index decreased serum insulin and glucose response, and HbA1c and Urinary C- peptide (a marker for insulin production) in both diabetic and nondiabetic subjects.

Fructose produces smaller postprandial insulin release than consumption of glucose-containing carbohydrate along with reducing circulating leptin levels as leptin production is regulated by insulin response to meals. This combined effect of lower insulin and leptin could lead to increased weight gain due to signalling to the brain suggesting still in need of food and hence increased food consumption, and since fructose is also preferentially metabolized to lipid in the liver, it could lead to insulin resistance (Elliott *et. al.,* 2002), which is possibly due to interference with insulin signalling (Lee *et. al.,* 1994). Exposure of the liver to large quantities of fructose stimulates lipogenesis and the accumulation of triglycerides contributes to decreased insulin sensitivity and hence hepatic insulin resistance (Hallfrisch, 1990). Wu and colleagues (2004) also state that there are long term negative effects of a high fructose diet which can lead to changes in digestion, absorption, plasma hormone levels (30% reduction in ghrelin, for example, as well as adiponectin), appetite and hepatic metabolism leading to the metabolic syndrome. A high fructose diet has also been shown to increase the amount of C-peptide and lower the number of insulin receptors in skeletal muscle and liver in mice, as well as decreasing the insulin stimulated

autophosphorylation in rats increasing of intracellular triglycerides stores leading to lipotoxicity and β-cell failure (Catena *et. al.,* 2003). Higashiura and colleagues (1999) showed a link between a high fructose intake in rats and the muscle fibre type with a change of Type 1 fibre (low twitch, Oxidative) to Type 2a fibre (fast twitch, Oxidative/Glycolytic), which could be reversed by the addition of a calcium antagonist. In a human study it was shown that a high fructose intake increased gluconeogenesis, total glucose output and glucose cycling and decreased the glucose rate of disappearance leading to insulin resistance (Dirlewanger *et. al.,* 2000). However, a small amount of fructose is beneficial in that its metabolite, fructose-1-phosphate increases glucokinase activity in the liver, which in turn, allows increased glucose sensing and suppression of gluconeogenesis (van Schaftingen, 1993).

### 1.2.2. Free Fatty Acids

An acute elevation of FFAs moderately stimulates insulin secretion in normal and elevated glucose conditions (Malaisse & Malaisse-Lagae, 1968; Crespin *et. al.,* 1969); however in Type 2 Diatetic individuals there is a long-term increase in FFAs, which may have severe effects on insulin secretion.

Obesity is characterised by increased levels of FFAs, and also by insulin resistance (Kolterman *et. al.,* 1980). Free Fatty Acids have a negative effect on insulin sensitivity in the liver and hence contribute to the production of

endogenous glucose, by inhibiting the acute insulin suppression of glycogenolysis and the elevated levels of plasma FFAs (Boden *et. al.,* 2002). Petersen and Shulman (2006) suggest that skeletal muscle insulin resistance is brought about by the loss of insulin activation of IRS-1 associated PI3K activity in high FFA environment and may be similar in the liver. Cortisol may also contribute to an increased lipolysis by further inhibiting the antilipolytic effect of insulin (Frayn *et. al.,* 1996). An increase of FFAs is released from abdominal adipocytes (abdominal obesity is a major predictor of insulin resistance and diabetes) into the portal system (Frayn *et. al.,* 1996), which inhibits insulin sensitivity.

Under normal physiological condition, FFAs sustain insulin release in the fasting individual and acutely enhance hormone release in the presence of glucose (McGarry & Dobbins, 1999); however, prolonged exposure to high concentrations of FFAs has a detrimental effect on β-cells (lipotoxicity) (Zhou & Grill, 1994; McGarry & Dobbins, 1999). A high level of FFAs reduce insulin release in response to glucose, suppresses proinsulin biosynthesis and decreases insulin stores (Bollheimer *et. al.,* 1998; McGarry & Dobbins, 1999), along with increasing the proinsulin to insulin ratio (Bjorklund & Grill 1999). Increased FFA levels have been shown to cause apoptosis of β-cells leading to the development of diabetes in mice (Lee *et. al.,* 1997; Shimabukuro *et. al.,* 1998), which is brought about by the caspase system along with other possible

proteases (Cryns & Yuan, 1998; Johnson, 2000; Lupi *et. al.,* 2002). The mechanism of apoptosis has not been fully elucidated but include the following possibilities: increased ceramide production, the activation of the transporter nuclear factor $_{k}$B, up regulation of iNOS, increased synthesis of NO, enhanced formation of NO-derived free radicals, DNA damage (Unger & Zhou, 2001; Lupi *et. al.,* 2002), the binding of FFA with PPARs is able to elicit several changes in the expression of many proteins, including some caspases (Chinetti *et. al.*, 1998) and there is a marked reduction in Bcl-2 mRNA, which is an apoptosis regulator gene product (Zamzami *et. al.*, 1998). The FFA-induced inhibition of glucose-stimulated insulin release may be related to the glucose-free fatty acid (Randle) cycle (Reviewed in Randle *et. al.*, 1988), by substrate competition, after their oxidation. Free Fatty Acid oxidation is inhibited by metformin (Patiane *et. al.*, 2000), which results in the increased glucose metabolism and insulin secretion, while FFA metabolism reduces the activity of pyruvate dehydrogenase, which alters glucose metabolism (Zhou *et. al.*, 1996). Lupi and colleagues (2002) have shown that a high level of plasma FFA decreases both glucose oxidation and utilization, which was more than could be accounted for by oxidation suggesting that other pathways are affected as well.

Free Fatty Acids induce insulin resistance by impairing the insulin-signalling pathway at the level of insulin-stimulated glucose transport or phosphorylation. Dresner and colleagues (1999) suggest that it is the

accumulation of metabolites other then fat, such as diacylglycerol (DAG), which results in insulin resistance. The increase in DAG results in a rise of protein kinase C (PKC), which is an enzyme that can phosphorylate serine and threonine residues on both the insulin receptor and the insulin receptor substrate along with the activation of the nuclear-factor-κB (NFκB) pathway (Itani *et. al.,* 2002). FFAs may also increase insulin resistance by increasing oxidative stress with both the PKC and the NFκB pathways being activated by reactive oxygen species (Griffin *et. al.,* 1999; Ceriello, 2000).

Free Fatty Acids are potent insulin secretagogues and can compensate for the insulin resistance that they can create with acute elevations stimulating insulin secretion and prolonged elevations potentiating glucose-stimulated insulin secretion (Crespin *et. al.,* 1973). However, in those who are first degree relatives of a Type 2 Diabetic individual, FFAs are unable to compensate for the insulin resistance that they create suggesting a genetic predisposition to pancreatic β-cell failure (Stogaard *et. al.,* 2003).

Peroxisome proliferator-activated receptor – gamma (PPAR-γ), which is one of the nuclear transcription factors that regulate the expression of numerous genes involved in lipid and carbohydrate metabolism, inflammation and vascular tone, is expressed at its highest concentration in adipose tissue and at a much lower level in the skeletal muscles and liver (Tontonoz *et. al.,* 1994). Peroxisome proliferator-activated receptor – gamma exerts it effect by lowering

plasma FFA levels, increasing plasma adiponectin levels, and redistributing visceral fat to subcutaneous depots similar to rosiglitazone (Mayerson *et. al.,* 2002). Free Fatty Acids are also natural ligands for peroxisome proliferator-activated receptor (PPAR-α), which is expressed in the liver, muscle, kidney and heart, which are the organs where fatty acids are oxidised and PPAR–α activation lowers plasma FFA levels through increased fat oxidation (Muoio *et. al.,* 2002). Specific PPAR-α agonists can lower lipid levels in rats and can improve their insulin sensitivity (Ye *et. al.,* 2001), however, human studies have shown either improved (Mussoni, *et. al.,* 2000) or no reaction to specific PPAR-α agonists (Asplund-Carlson, 1996) A diet high in saturated fats has also been implicated in a reduction in the number, location, and morphology of mitochondria which is strongly associated with insulin resistance, due to a reduction in the expression of genes involved in the oxidative capacity of the cells such as those of the electron transport chain, mitochondrial protein genes and those involved in mitochondrial biogenesis (Sparks *et. al.,* 2005; Ukropcova, *et. al.,* 2007). The healthy individual, with normal body fat content relies on lipids as their main source of energy during the fasting state and can switch to glucose oxidation with introduction of insulin, while in the insulin resistant state the switch to glucose oxidation does not occur due to decreased mitochondrial oxidative enzyme activity response to insulin (Ukropcova *et. al.,* 2007).

### 1.2.3 Genetics

One or more lesions in a number of different genes have been shown to predispose an individual to Type 2 Diabetes and in particular Maturity Onset Diabetes of the Young (MODY), which is a subset of the Type 2 Diabetes, however, they generally remain silent. One gene that has been of particular interest is for the apolipoprotein B, which influences parameters related to insulin resistance, with its polymorphisms being silent and so the effects must be mediated through linkage to other genes (Bentzen *et. al.,* 2002). Kovacs and colleagues (2002) have implicated the ORP 150 gene as a possible link for Type 2 Diabetes in the Prima Indian population, who have a very high incidence of Type 2 Diabetes, hypothesizing that it plays some role in responding to glucose levels by altering muscle glucose uptake. Mutations in the gene WFS1 have been shown to produce β-cell death in a form of diabetes known as Wolfram Syndrome and Minton and colleagues (2002) have shown that variations of this gene may lead to susceptibility to Type 2 Diabetes. Siitonen and colleagues (2006) have shown that a sequence variation in a gene encoding for the adiponectin receptor 1 has implications for body size and plasma insulin levels although they appear to be dissociated from each other. The Pro12 allele of the PPAR-γ gene, which, when combined with low physical activity leads to a higher risk of the disorder (Nelson *et. al.,* 2007). Genetic variation of the adiponectin locus is a predictor of adiponectin levels and hence insulin

resistance and there have been several polymorphism linked to Type 2 Diabetes (Menzaghi *et. al.,* 2007). Also a haplotype of the CRP gene has been shown to result in a high serum CRP level, and the incidence of diabetes was much higher amongst this group of individuals than those individuals who had the three other common haplotypes. This indicates a possible genetic role in the development of diabetes (Dehghan *et. al.,* 2007).

MODY is a monogenic dominantly inherited form of Type 2 Diabetes that is characterised by defective insulin secretion and mutations in five genes have been implicated in this including the hepatocyte nuclear factor-4α gene and the hepatocyte nuclear factor-1α gene (Yang *et. al.,* 2002).

First degree relatives of the Type 2 Diabetic patient will often exhibit symptoms of the pre-diabetic state or metabolic syndrome suggesting that genetics plays a role in the development of the disease. Karlsson and colleagues (2006) have suggested that the insulin-mediated glucose uptake from glucose tolerant individuals who are first degree relatives of diabetic subjects is impaired and this may lead to impaired glucose tolerance and later to Type 2 Diabetes.

### 1.2.4. Uterine Environment

There is considerable evidence to show that the intrauterine environment of a foetus can predispose an individual to diabetes, with the higher the birth-weight, which is a measure of the intrauterine environment in humans, the less

likelihood of a person getting Type 2 Diabetes (Birgisdottir *et. al.,* 2002), indicating the need for high quality nutrition by the expectant mother is important in the prevention of Type 2 Diabetes. The intrauterine environment is argued to change gene expression and lead to physiological or morphological phenotypes associated with diabetes (Eriksson *et. al.,* 2002), suggesting there is a link between the Pro12Pro genotype of the PPAR-γ2 gene being associated with higher fasting insulin concentrations and insulin resistance in babies with a low birth weight. Intrauterine growth failure leading to a small birth weight is also shown to affect the number of pancreatic β-cells as well as the function of these cells (Eriksson *et. al.,* 2002). Lawlor and colleagues (2006) showed that poor intrauterine growth and premature births were associated with the risk of developing diabetes in later life, though no understanding of why this occurs has been elucidated, while Singh and colleagues (2006) showed that children of diabetic mothers were more likely to develop Type 2 Diabetes then if the father was diabetic and suggest that this may be the result of mild hyperglycaemia within the uterine environment, reducing β-cell function.

Insulin-like growth factor-1 (IGF-1) and insulin are essential foetal growth factors (Hill *et. al.,* 1998) and is also important for growth and development of β-cells (Vaessen *et. al.*, 2001) and Vaessen and colleagues (2002) have shown that a mutation in the IGF-1 gene and the GK gene lead to both a predisposition to Type 2 Diabetes and low birth weight.

Early postnatal malnutrition can have major consequences on fetal and postnatal development in both humans and animals (Gluckman and Harding, 1992; Owens, 1991). Protein/calorie malnutrition in particular or protein deficiency only *in utero* in the mother will lead to decreased β-cell mass or islet volume in off-spring which may be due to a decreased islet neogenesis with prolonged maternal malnutrition until the end of the lactation period resulting in up to 70% decrease in β-cell mass. This was not restored at age by normal feeding after weaning in animals (Blondeau *et. al.,* 1999). Insulin stores and fasting insulin is also decreased in previously malnourished mice and there is an impaired secretory response to glucose (Blondeau *et. al.,* 1999).

The mechanism linking low birth rate and glucose intolerance has not yet been elucidated, however, one suggestion has been that poor nutrition *in utero* causes the foetus to become thrifty at a time when β-cell development proceeds more rapidly, resulting in impaired growth of these cells and, hence, leads to a predisposition of diabetes later in life (Blondeau *et. al.,* 1999). Interestingly, this has been shown to lead to decreased plasma insulin but normal plasma glucose (Garofano *et. al.,* 1998), also maternal protein deficiency does not lead to impaired glucose tolerance in the adult offspring (Holness and Sugden, 1996).

### 1.2.5 Inflammation

Generalised non-specific chronic inflammation indicated by high levels of inflammatory markers have been linked with Type 2 Diabetes, with higher than normal levels of CRP and other inflammatory markers being shown to be present in conjunction with Type 2 Diabetes (Festa *et. al.,* 2002; Freeman *et. al.,* 2002). van Exel and colleagues (2002) hypothesize that there is a pro-inflammatory response in patients with Type 2 Diabetes and suggest that this is under genetic control. Research by Mansfield and colleagues (1996) showed an increase in inflammatory markers in first-degree relatives supporting the genetic theory with one study showing that there is a four-six fold increased risk in non-diabetic subjects to develop Type 2 Diabetes when there were inflammatory markers present (Festa *et. al.,* 2002).

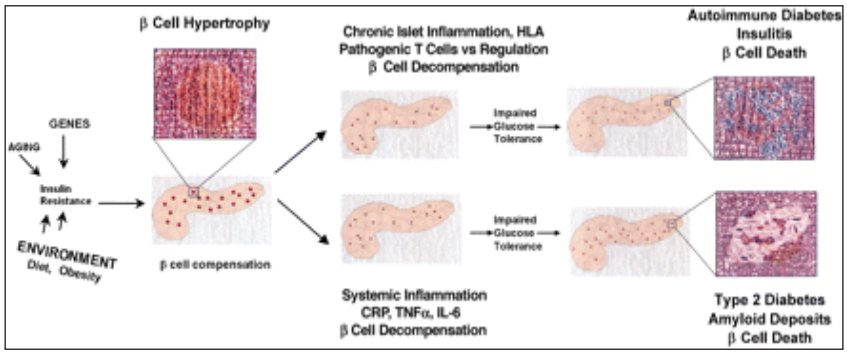



Figure 1.8: Relationship between islet autoimmunity and systemic inflammation in adult patients with diabetes. (Pietropaolo *et. al.,* 2007).

Beta-cell hypertrophy is an adaptation to insulin resistance and is associated with the initial stages of insulin resistance. This is followed by chronic

inflammation of the islets due to an imbalance between pathogenic and regulatory T-cells in Type 1 Diabetes, while the activation of the acute phase systemic inflammatory response through an array of plasma proteins and cytokines, leads to Type 2 Diabetes (Pietropaolo *et. al.,* 2007).

Elevated CRP has been associated with obesity, insulin resistance and hyperglycae mia (Ford, 1999; Hak *et. al.,* 1999; Visser *et. al.,* 1999), suggesting that these disorders may be the result of an ongoing acute phase response, reflecting a chronic adaptation of the immune system (Pickup *et. al.,* 1997). CRP is a sensitive acute phase marker of inflammation (Chambers *et. al.,* 1991), however, its function is not well defined. It binds to damaged tissue and nuclear antigens in a calcium-dependent manner, activating complement and generating proinflammatory cytokines suggesting a proinflammatory role (Clos, 2000).

Obese patients showed a generally higher CRP level due to the release of Il6 from the adipocytes, which stimulate hepatic CRP production (Liu *et. al.,* 2002); however, McLaughlin and colleagues (2002) have shown that the relationship between plasma CRP levels and insulin resistance are independent of obesity even though the CRP levels are predominately elevated in individuals who are obese and have insulin resistance and the correlation appears to be stronger in women than men (Thorand *et. al.,* 2007).

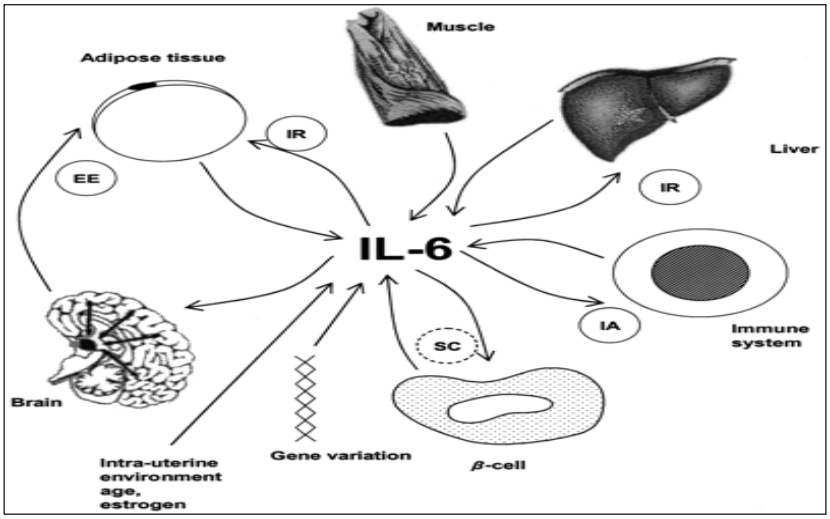



Figure 1.9: Model of IL-6 actions of potential relevance for the pathogenesis of type 1 and type 2 diabetes. IL-6 is produced by many inflammatory cells, adipose tissue, working muscle, and even the ß-cell. Expression is determined by genetic variation, intrauterine environment, age, and sex steroids. IL-6 induces insulin resistance in adipose tissue and liver and may synergize with proinflammatory cytokines to produce ß-cell damage. IL-6 also regulates energy expenditure probably by the effect on brown adipose tissue via effects on the CNS. EE, energy expenditure; IA, immune activation; IR, insulin resistance; SC, synergy with proinflammatory cytokines (Reviewed in Kristiansen and Mandrup-Poulsen, 2005).

Interleukin-18 (IL-18) has also been implicated in the development of Type 2 Diabetes. Elevated levels of IL-18 and CRP and IL-18 and IL-6 have been shown to be present in Type 2 Diabetic patients independent of a generalised pro-inflammatory state (Thorand *et. al.,* 2005) and IL-6 is an independent marker for insulin resistance independent of gender (Thorand *et. al.,* 2007); however, increased levels of IL-6 does not appear to have any effect on β-cell function or insulin resistance in muscle. There is some evidence that IL-6 may cause insulin resistance in hepatocytes and impaired insulin signalling in

adipocytes, as well as impaired action within the Central Nervous System centres involved in energy metabolism, resulting in obesity, which in turn leads to insulin resistance (Reviewed in Kristiansen and Mandrup-Poulsen, 2005). The IL-6 receptor uses the Janus Kinases (JAKs) as intracellular pathways which may lead to a decrease in the insulin signalling, possibly via the participation of protein kinases and tyrosine phosphatase activation (Kroder *et. al.,* 1996) or SOCS interaction (Ricusset *et. al.,* 2004) with the insulin receptor. Engstrom and colleagues (2005) have shown that Complement C3 and C4, which are major plasma proteins of the immune system complement pathway and are increased in response to inflammation at a slower rate then the more acute phase proteins, have a substantial correlation with obesity and hence show a possible link with the increased risk of developing Type 2 Diabetes.

### 1.2.6. Oxidative Stress

Oxidative stress has been linked to the pathogenesis of Type 2 Diabetes and its complications, and stress proteins have been shown to protect against oxidative stress. There is a decrease in the heat shock proteins in the muscles of patients with Type 2 Diabetes and it has been hypothesized that this may be a link between insulin resistance and Type 2 Diabetes (Kurucz *et. al.,* 2002), although at this stage the mechanism is not understood. Ferandez and colleagues (2002) have suggested that the link may be that a bi-directional-iron affects glucose metabolism and glucose metabolism affects several iron

metabolic pathways, showing that increased iron stores predict Type 2 Diabetes while iron depletion is protective, suggesting that increased levels of iron may be toxic to cells and also cause inflammation. Evans and colleagues (2003) have proposed that there is a hyperglycaemia-induced, and possibly a FFA induced, activation of stress pathways and the production of reactive oxygen and nitrogen species, that leads also to the development of complications related to both Type 1 and Type 2 Diabetes and also the development and progression of insulin resistance and impaired insulin secretion. Type 2 Diabetic patients are also reported to have reduced muscle oxidative enzymes and have shown an increase in muscle fatigability (Halvatsiotis *et. al.,* 2002). This could lead to a decrease in the ability of insulin to stimulate glucose uptake.

### 1.2.7. Microvascular Disease

Type 2 Diabetes has been shown to lead to microvascular disease, however, some studies suggest that the microvascular disease appears first and may contribute to the pathogenesis of the diabetic state (Hsueh & Law, 1998; Tooke, 1995). Morris and colleagues (1995) demonstrated microvascular abnormalities (impaired microvascular reactivity and flow in the skin and skeletal muscles) in people with Type 2 Diabetes and in people at high risk of developing diabetes, such as those with impaired glucose tolerance and first degree relatives of persons with diabetes (Caballero *et. al.,* 1999; Balletshofer *et. al.,* 2000). It has been suggested that the microvascular alterations may result in a reduced ability

of insulin to mediate glucose uptake in skeletal muscles (Wong *et. al.,* 2002); the alterations may lead to inflammation, which has been linked to diabetes.

Metabolic abnormalities associated with increased platelet sensitivity to pro-aggregants and decreased platelet sensitivity to endogenous anti-aggregants has been associated with Type 2 Diabetes (Vinik *et. al.,* 2001; Beckman *et. al.,* 2002).

Hematocrit has been positively correlated with insulin resistance and many of the symptoms associated with the metabolic syndrome (high blood pressure, elevated cholesterol, and serum triglycerides, and low HDL) (Tamariz *et. al.,* 2003; Nakanishi *et. al.,* 2004). Although the correlation is not known, it has been suggested that as hematocrit is a major determinant of blood viscosity (MacRury and Lowe, 1990) and when this is elevated it reduces the blood flow to skeletal muscle, therefore interfering with insulin-mediated glucose uptake in skeletal muscle (Baron *et. al.,* 1994).

### 1.2.8. Obesity

The association between obesity and Type 2 Diabetes has been accepted for sometime, and although progress has been made, the exact mechanisms have not been established (Reviewed in Kahn *et. al.,* 1996b). Adipose cells secrete FFAs, as products of triglyceride metabolism and are the main form in which energy is transferred from adipose tissues to other sites for metabolic use and have also

been implicated in the induction of insulin resistance in tissues other then adipose tissues (Boden, 1999), by the release of numerous peptides such as resistin.

An important aspect of obesity is the placement of the adiposity where subcutaneous and visceral abdominal fat areas are linked to Type 2 Diabetes whereas other fat stores are not (Taniguchi *et. al.,* 2002); However whole body obesity and waist circumference and waist to hip ratio, both an indicator of central obesity, are all strongly related to the risk of the development of diabetes (Meisinger *et. al.,* 2006a).

Sterol regulatory element binding protein (SREBP-1) is a transcription factor, which plays many important roles in fatty acid metabolism and adipogenesis, which is positively modulated by insulin. One SREBP-1 isoform is commonly seen in adipose tissue of the obese and Type 2 Diabetes, which may be a possible link between diabetes and obesity (Sewter *et. al.,* 2002).

Hampton (2004) states that there appears to be a vicious cycle where an obese insulin-resistant woman, has an obese foetus *in utero* which remains obese throughout its life, has a greater risk of developing Type 2 Diabetes.

### 1.2.9. Lifestyle

A sedentary lifestyle has for decades been linked with diabetes, however, the mechanism has been unclear, although it may be due to inhibition of GLUT4

translocation. Many other lifestyle factors have also been implicated in Type 2 Diabetes such as smoking where it has been shown that there is an increased level of insulin resistance occurring in smokers with and without diabetes (Targher *et. al.,* 1997; Gunton *et. al.,* 2002; Foy *et. al.,* 2005). Gunton and colleagues (2002) have shown that cessation of smoking will bring about an improvement in glycosylated haemoglobin ($HbA_{1c}$) levels, which may be due to a change in many lifestyle factors at the same time, or it may be a direct effect and the improvement parallels the UKPDS metformin group (Gunton *et. al.,* 2002). Meisinger and colleagues (2006b) have shown that there is a significant dose-dependent relationship between smoking and in particular the nicotine and tar intake and the risk of developing Type 2 Diabetes in men; however, the study was less convincing with women but this may have been due to the low number of women in the cohort. Smoking is known to produce inflammation and this itself appears to play an important role in the development of diabetes (Rimm *et. al.,* 1993; Schmidt *et. al.,* 1999; Manson *et. al.,* 2000; Pradham *et. al.,* 2001).

Caffeine, an adenosine receptor antagonist, when ingested decreases glucose uptake suggesting the role of adenosine in glucose disposal and a decrease in carbohydrate storage (Greer ***et. al.,*** 2001). As coffee is a common beverage amongst the Western world where Type 2 Diabetes is epidemic, advice given to people as to the possible connection between caffeine ingestion and Type 2 Diabetes might be a way of decreasing the incidence of this disease in

the future. However, a study undertaken by van Dam and colleagues (2004) showed that habitual, chronic coffee consumption may reduce the risk of impaired glucose tolerance and a lower incidence of Type 2 Diabetes, but was not associated with FBGL or impaired fasting glucose, suggesting that coffee affects post-load rather then fasting glucose metabolism through increased insulin sensitivity. They also showed that coffee consumption was cross-sectionally linked with lower values for markers of insulin resistance such as inhibition of g6pase activity, decrease in absorption rates by inhibiting the sodium dependent transporters, and a reduction on oxidative stress, all possibly due to the chlorogenic acid within the coffee.

The consumption of soft drinks has become a major problem in many countries, as the high sugar intake has led to an increase in obesity, which in turn has led to an increased risk of diabetes. Consumption of fruit punch has also been implicated in this increased risk (Spurgeon, 2004). Sugar-sweetened soft drinks now contribute 7.1% of total energy intake and represent the largest single food source of calories in the USA (Block, 2004). The rise in obesity and Type 2 Diabetes parallels the increase in sugar-sweetened soft drinks (Bray ***et. al.,*** 2004), and hence programs to decease the amount of sugars in drinks or using alternate sweetening agents may decrease this ever growing problem. As fruit juices do not tend to have the same risk factors associated with Type 2 Diabetes (Schulze *et. al.*, 2004), possibly due to them having a lower glycemic

index, and more soluble fibre and possibly other chemicals that can be beneficial (antioxidants being a major consideration). There also needs be educational programs to inform and encourage people to alter their choice of beverage/drinking habits.

There has been a considerable increase in the eating of fast-foods and a study was undertaken by Pereira and colleagues (2005) to determine if there was any correlation between this increase and the increase in Type 2 Diabetes and they suggested that fast food promotes a positive energy balance leading to obesity and related diseases, due to the large portion sizes generally served in fast food outlets; palatability, emphasizing taste preferences of sugar, salt and fat; high energy density and the high glycemic load foods being offered. After a 15 year follow-up on several black and white, male and female Americans it was observed that eating the average fast-food diet by this population showed a strong, positive and independent correlation with obesity and insulin resistance (Pereira ***et. al.,*** 2005).

### 1.2.10 Lung Vital Capacity

A low lung vital capacity has been linked to the development of impaired glucose tolerance, insulin resistance and Type 2 Diabetes in later years. Possible mechanisms include hypoxemia and inflammation during foetal and early childhood (Yeh *et. al.,* 2005). Hypoxemia results in increased sympathetic activity which has been shown to increase the risk of Diabetes; however, this has

been debated (Yeh *et. al.,* 2005). Inflammation has been linked with diabetes and decreased lung function will often result in inflammation as seen in lower respiratory tract infection, however this link has not been proven either (Yeh *et. al.,* 2005). Low vital capacity may also interfere with organ development and the metabolic pathway programming, which, in addition to a genetic predisposition could lead to insulin resistance (Yeh *et. al.,* 2005).

## 1.3. ORTHODOX TREATMENT

Therapeutic options that are currently available for the treatment of Type 2 Diabetes include dietary modifications, lifestyle modifications, oral hypoglycaemic agents and insulin. In early Type 2 Diabetes dietary and lifestyle modifications are usually the only treatment recommended, especially if there is a weight problem as well. In many cases this will control the BGLs, however, when diet modifications no longer exert the required effect, oral hypoglycaemic agents become very important in the treatment of Type 2 Diabetes.

### 1.3.1 Diet and Lifestyle

Obesity has been associated with insulin resistance and Type 2 Diabetes, therefore reducing caloric intake improves glycemic control. Caloric intake is more important than the actual weight loss, which will improve both insulin resistance and impaired insulin secretion (De Fronzo, 1988) as once weight loss

has occurred, even without weight gain, metabolic control can deteriorate again because during caloric restriction there is a rapid fall in post-absorptive endogenous glucose production (Henry *et. al.,* 1986).

Carbohydrates are the cause of increases in blood glucose but it is important that they are still consumed throughout the day as they are the main fuel for the body and are the primary fuel for the brain and central nervous system. Due to this low-carbohydrate diets are not recommended in the management of diabetes and it is recommended that 45-65% of calories are derived from carbohydrates with a minimum intake of 130 gm per day for adults (Institute of Medicine of the National Academies, 2002). Since both the glycemic index and the amount of carbohydrate consumed have an effect on the blood glucose level it was found that decreasing the glycemic index of diet reduced postprandial plasma glucose by the same amount as reducing the carbohydrate intake (Wolever and Mehling, 2003). Therefore patients with Type 2 Diabetes should increase foods which are low glycemic and high fibre over those that are high glycemic and low fibre. These finding were supported by Lindstrom and colleagues (2006) in the Finnish Diabetes Prevention Study, where they showed that high-fibre, low-fat diets led to long term weight loss and a decrease in the risk of Type 2 Diabetes, in those who had impaired glucose function.

The eating of foods high in natural fibre is also important with one study looking at the Mediterranean Diet showing that natural fibre foods and also foods that had fibre added both decreased the absorption of glucose from the intestine but only the natural fibre foods kept plasma glucose levels low (Riccardi *et. al.,* 2003). The mechanism of this was not elucidated, however, it was noted that that postprandial plasma acetate was much higher in the natural fibre rich diets. A diet high in soluble fibre will reduce the need for insulin (Landin *et. al.,* 1992) and improve glycaemia (Vuksan *et. al.,* 2000a). Studies with purified viscous fibres demonstrated a reduced rate of gastric emptying (Holt *et. al.,* 1979; Leeds, *et. al.,*1981) and small intestine absorption (Blackburn *et. al.,* 1984; Floune *et. al.,* 1984) resulting in a reduced blood glucose and insulin response. Levels of insulin and GIP are found to be reduced with a high fibre diet (Jenkins *et. al.,* 1977; Jenkins *et. al.,* 1979; Jenkins *et. al.,* 1980). The mechanism of action appeared to be the slowing down of absorption of glucose rather then a malabsorption (Jenkins *et. al.,* 1977; Jenkins *et. al.,* 1979). High fibre foods that also have a low glycemic index have shown the greatest improvement in decreasing HbA1c levels (Simpson *et. al.,* 1981). Suzuki and colleagues (2002), in a study looking at fruit and vegetable intake in a Japanese population, suggested that such a diet, rich in antioxidants, might be protective against Type 2 Diabetes. Weickert and colleagues (2006) showed that purified insoluble fibre, the predominant fraction of cereal fibre, and consisting of cellulose and hemicellulose, improved insulin sensitivity if consumed within the

daily recommended range. This improvement occurred within three days of taking the fibre; however, the mechanism of action does remain unexplained. Weickert and colleagues (2006) showed that the increased insulin sensitivity was not due to the fermentation process within the colon or an increase in the amount of GLP-1 produced. Qi and colleagues (2006) have suggested that dietary cereal fibre increases the circulating adiponectin levels which may be effective in lowering blood glucose levels. Gentilcore and colleagues (2006) showed that a small amount of olive oil 30 minutes prior to a carbohydrate meal decreased gastric emptying, rise of blood glucose and insulin levels, however it does stimulate the secretion of GLP-1 and GIP thus delaying the onset of postprandial rise in glucose levels, however, this did not occur to such an extent if the olive oil was taken with the meal.

Liu and colleagues (2002) showed that the quality and quantity of carbohydrate intake was directly related to plasma concentrations of CRP, with a high dietary glycemic load being proportional to the plasma CRP indicating that a high glycemic load may affect insulin resistance through a proinflammatory response. O'Riodain and colleagues (1995) showed that elevated concentrations of insulin and counter-regulatory hormones are directly associated with hepatic production of CRP. Thomas and Wolever (Reviewed, 2003) have shown that reducing carbohydrate intake increases postprandial FFAs but does not improve blood glucose levels, however, low glycemic foods do lower both FFAs and

glucose levels. Therefore, it is strongly suggested that a high-carbohydrate but low glycemic foods be chosen in the diet.

High fat diets have been linked to diabetes and its complications; however it appears that the type of fat that is eaten may play a major role in these complications. One study using db/db mice has shown that diets high in saturated fats tend to decrease adiponectin levels and increase inflammation in white adipose tissue; however, the use of n-3 series marine polyunsaturated fats over a prolonger period will return the adiponectin levels to normal and will assist in the decrease of inflammatory markers such as interleukin-6 (Todoric *et. al.,* 2006).

Diet is better at controlling BGL if accompanied by exercise, which has been shown to improve both glucose effectiveness, the ability of hyperglycaemia to promote glucose disposal at basal levels of insulin, and insulin sensitivity (Nishida *et. al.,* 2001). Di Loreto and colleagues (2005) have shown that it is necessary to reach certain energy expenditure levels through exercise for it to be effective in weight loss and also the decrease of FBGLs. An increase in energy expenditure is beneficial only to a certain point where any increased energy expenditure does not appear alter FBGLs, blood pressure, cholesterol or triglycerides and Jeon and colleagues (2007) have shown that moderately intensive physical active is all that is needed to reduce the risk of Type 2 Diabetes; however Sriwijitkamol and colleagues (2007) have suggested

that the Type 2 Diabetes, who is also obese need to exercise at a higher intensity to stimulate the enzyme AMPK and the Akt substrate AS160 to the same level as that of the non-obese individual. These benefits occurred in the absence of weight loss so it appears that the important aspect is the aerobic physical activity, though it is noted that it still may be a result of the change of the composition of body tissues with the build up of muscle and loss of adipose tissue (Di Loreta, 2005). Exercise has also been shown to decrease the circulating levels of Il6 in both lean and obese individuals with and without diabetes and as chronic inflammation has been linked to diabetes this may be one way in which an exercise program will also be of benefit (Dekker *et. al.,* 2007). One area of major concern today is the epidemic of early-onset Type 2 Diabetes and Burns and colleagues (2007) have shown that these individuals, who are generally obese, are severely insulin resistant and have significant loss of β- cell function when compared with control subjects matched for age and obesity; however, neither group responded metabolically to aerobic exercise training. It has been suggested that this might be due to a susceptibility genotype or a mitochondrial dysfunction due to chronic lipotoxicity or hyperglycaemia.

### 1.3.2. The Sulfonylurea Compounds

The sulfonylurea compounds, such as Glibenclamide, Tolbutamide and Glipizide, have been available for sometime and used successfully in the

treatment of Type 2 Diabetes (Hellman & Taljedal, 1975; Ashcroft & Ashcroft, 1992), as they exert glucose-like action on the $K^+$ATP channels by closing them and decrease the $K^+$ permeability of the β-cell membrane. This leads to the depolarisation of the cell, initiation of the voltage-gated $Ca^{2+}$ influx, and insulin release (Sturgess *et. al.,* 1985; Zunkler *et. al.,* 1988). Sulfonylurea's also enhance exocytosis through enhancement of protein kinase C-dependent exocytosis, and is additive to the cAMP-dependent effects (Eliasson *et. al.,* 1996). It leads to a greater expression of GLUT1 (Tordjman *et. al.,* 1989; Wang *et. al.,* 1989; Tsiani *et. al.,* 1995), which enhances basal glucose uptake, these medications also have an acute effect on glucose utilization in fat and muscle. The sulfonylurea's have a well-characterised profile of side effects, especially those of a gastric nature and, also, and very importantly hypoglycaemia, which could be fatal (Prout, 1974). Long-term administration may over stimulate β-cells, which could lead to an even greater β-cell dysfunction as apoptosis occurs (Goodman, 2001) though Holman (2006) suggests that they neither increase or decrease β-cell apoptosis, as well as impairing endothelial function leading to increased risk for ischemic complications (Del & Pulizzi, 2006). They will bring about normalisation of the BGL; however, they do not appear to prevent the micro and macro vascular effects of diabetes due to its inability to maintain a normal BGL for an extended period of time (Turner *et. al.,* 1999). Also more of the drug is needed to maintain the homeostatic effect on glucose, as treatment

progresses, as there appears to be a development of tolerance to it or often other groups of drugs need to be added to return the BGL level to normal.

### 1.3.3. The Biguanides

Biguanides, for example, Metformin, are another class of hypoglycaemic agents that are in common use today as they do not promote insulin release but act by increasing glucose utilization in the intestines (Penicaud *et. al.,* 1989), reducing hepatic glucose output (Meyer *et. al.,* 1967; Jackson *et. al.,* 1987) by, possibly, interrupting mitochondrial oxidative processes (Kirpichnikov *et. al.,* 2002) and increasing peripheral glucose utilization (Hother-Nielson *et. al.,* 1989 Galuska *et. al.,* 1994; Fisher *et. al.,* 1995). The increased uptake of glucose is independent of insulin and is associated with GLUT4 and GLUT1 translocation to the plasma membrane without affecting the number present (Kozka & Holman, 1993; Fisher *et. al.,* 1995). Musi and colleagues (2002) showed that metformin increased AMP-activated protein kinase activity in skeletal muscle of patients with Type 2 Diabetes, bringing about a higher rate of glucose disposal and glycogen concentration. However, it does not affect insulin binding or insulin receptor kinase activity (Fantus & Brosseau, 1986). Rajala and colleagues (1998) noted that many Type 2 Diabetic patients have microvascular disease before they are diagnosed with Type 2 Diabetes. Turner and colleagues (1999) showed that Metformin did not reduce these defects.

### 1.3.4. The Thiazolidinediones

The Thiazolidinediones (TZDs) are a class of antidiabetic drugs, which enhance insulin sensitivity in muscle and adipose tissues (Henry, 1997), by acting as a high affinity ligand for the nuclear receptor PPAR- γ (Chawla *et. al.,* 1994; Lehmann, 1995). PPAR-γ acts as an adipogenic determination factor (Barak, 1999; Rosen, 1999), and the TZDs bind to PPAR-γ and hence lower BGL but also increase insulin sensitivity through non-TZD PPAR-γ ligands (Mukherjee, 1997; Wilson *et. al.,* 2000). TZDs act in a way similar to nicotinic acid in decreasing plasma FFAs, however, without the rebound effect (Chen *et. al.,* 1999). Petersen and Shulman (2006) suggest that TZDs increase insulin sensitivity in the adipocyte which results in more efficient storage of fat, which in turn, results in a redistribution of fat away from the intracellular levels in the hepatocyte and myocyte. TZDs also play a major role in the development of adipocytes (Tontonoz *et. al.,* 1994a), and it has been postulated that they improve insulin sensitivity by redistributing fat from visceral to subcutaneous fat (de Souza *et. al.,* 2001) and by increasing plasma adiponectin levels (Boden, 2003). TZDs have also been shown to decrease TNF-α, a pro-inflammatory cytokine produced in adipocytes and plasma CRP levels and increases the level of adiponectin, which has been shown to increase insulin sensitivity (Staels and Fruchart, 2005; Hung *et. al.,* 2006). Kubota and colleagues (2006) have shown that the TZDs work in acting both on adiponectin-dependent and independent

pathways in decreasing blood glucose levels, with lower dosages increasing adiponectin levels and acting on the liver and higher doses improving adiponectin-independent pathways in skeletal muscle.

Repaglinide, a potent insulin secretagogue that acts on the ATP-sensitive potassium channels on the β-cell plasma membrane through a distinct binding site (Gromada ***et. al.*,** 1995). Repaglinida is important as it works synergistically with metformin in poorly controlled Type 2 Diabetes, obese patients and can be substituted for metformin in those who are experiencing gastrointestinal side effects, without effecting glycaemic control. Cerivatatin, one of this group of drugs, improves first phase insulin secretion and increased insulin-mediated glucose uptake (Paniagua ***et. al.*,** 2002).

## 1.4 NATURAL THERAPIES AND SUPPLEMENTS

There are many natural therapies and supplements used in the treatment of diabetes, including ones from India, China, African, South American, Middle Eastern and Western traditions. Many herbs, for example, *Achyrocline satureiodes* (Lam.) DC. (Asteraceae) (Carney ***et. al.*,** 2002), *Ceiba pentandra*, (Ladeji, 2003), *Hypoxis hemerocallidea* Corm (Hypoxidaceae), (Mahomed and Ojewole, 2003) and *phyllanthus amarus* (Raphael ***et. al.*,** 2002) have had exploratory work carried out on them and have been shown to decrease FBGLs without actual mechanisms being elucidated at this stage. While others such as

*Polygonatum sibiricum* (Bensky & Gamble, 1993) have been used by different folklore medicine groups but there is no evidence of effectiveness, or toxicity. Grant and colleagues have suggested that although there is some positive evidence for the use of Chinese herbs for the treatment of Type 2 Diabetes, there is a lack of trials with relevant details of methods, randomisation, and poor reporting and other possible bias.

Different herbs work in different ways in reducing BGLs, with some only being effective in early stages of Type 2 Diabetes, while others are only effective if taken in certain amounts and others, if taken at certain times. Often in traditional medicine there is a combination of herbs prescribed due to the claims that the combination will act synergistically, enhancing the effect of the single herb, or act to decrease side effects of the herbs. However, this has not been shown to be efficacious at this stage and much more work needs to be carried out to show the effectiveness of this.

Glucose uptake by skeletal muscle, liver and adipocytes is decreased in Type 2 Diabetes, and insulin resistance occurs due to a down-regulation in the number of insulin receptors and/or the amount of GLUT4 and its translocation. There are several herbs, such as *Bidens pilosa* (Alarcon-Aguilar *et. al.*, 2002), *Gymnea sylvestre* (Persaud *et. al.*, 1999; Shane-McWhorter, 2001), *lyophyllum decastes* (Miura *et. al.*, 2002), *Panax Ginseng* (Suzuki, 1989) and *Scoparia dulcis* (Pari & Venkateswaran, 2002b) which have been shown to upgrade

GLUT4 production and translocation and/or increase the number of insulin receptors on cells and, hence, aid in the maintenance of BGLs.

The amount of insulin secreted when stimulated by increased BGLs due to glucose absorption or gluconeogenesis is also very important in maintaining glucose homeostasis. The amount of insulin being secreted can be adjusted by the amount stored in vesicles, the production of more insulin and also the maintenance and regeneration of the β-cells. Some herbs have been shown to act in these ways to decrease BGLs, including *Barleria lupulina* (Suba *et. al.*, 2004), *Cyclocarya paliuris* (Kurihara *et. al.*, 2003), *Gymnema sylvestre* (Shanmugasundaram *et. al.*, 1990), *Nigella sativa* (Kanter *et. al.*, 2003) and *Pterocarpus marsupium* (Hii & Howell, 1984).

Gluconeogenesis, or the production of glucose in the liver to maintain fasting BGLs is important, as some tissues such as the brain and kidneys can only receive energy from this source. However, in Type 2 Diabetes this is up regulated and too much glucose is produced. Many herbs, such as *Cassia auriculate* (Latha & Pari, 2003a), *Gongronema latifolium* (Ugochukwu & Babady, 2003a), *Stevia rebaudiana* (Malaisse *et. al.*, 1998) and *Trigonella foenum graecum* (Vats *et. al.*, 2003) act in normalising the enzymes of gluconeogenesis thus decreasing the amount of glucose produced, and, hence the BGLs.

*Glycyrrhiza uralensis* has been shown to exhibit PPAR-γ ligand-binding activity and contains a phenolic Glycyrin, that appears to bind with PPAR-γ receptors (Kuroda *et. al.*, 2003), which are found in insulin-responsive tissues to modulate one or more steps in the insulin-signalling pathway (Steppan *et. al.*, 2001a) and thereby decreases BGLs.

The rate of absorption of glucose from the gastrointestinal tract will also influence BGLs, due to the fact, that if glucose is absorbed slowly and at a more even rate, then there is less fluctuation in BGLs. Fibre is one of the best known ways of slowing glucose absorption (Vuksan *et. al.*, 2000a). Some herbs that also slow down glucose absorption include, *Hypoxis hemerocallidea* (Mahomed and Ojewole, 2003), *Momordica charantia* (Chopra *et. al.*, 1958) and *Morus indica* (Andallu & Varadacharyulu, 2002) along with many everyday fruits and vegetables. Other herbs, such as *Casearia esculenta* (Joglegar *et. al.*, 1959), and *Cyclocarya paliuris* (Kurihara *et. al.*, 2003) also appear to decrease glucose absorption from the gastrointestinal tract, however, the mechanism has not been elucidated at this stage.

Inflammation has been linked to Type 2 Diabetes although the exact mechanism is not known at the moment. Some herbs such as *Curcuma longa* (Arun & Nalini, 2002), *Scrophularia deserti* (Ahmed *et. al.*, 2003) and that *Zingiber officinale* (Akhani *et. al.*, 2004) have been shown to exhibit anti-inflammatory properties and this may be the way that they decrease BGLs.

Herbs, such as *Brassica juncea* (Yokozawa *et. al.*, 2003; Yokozawa *et. al.*, 2002), *Camellia sinensis* (Mustata *et. al.*, 2005), Cinnamon (Anderson *et. al.*, 2004), *Curcuma longa* (Arun & Nalini, 2002), *Phyllanthus amarus* (Raphael *et. al.*, 2002) and of *Zingiber officinale* Roscoe (Zingiberaceae) (Akhani *et. al.*, 2004) may aid in the management of Type 2 Diabetes by their anti-oxidant effect, with many herbs decreasing oxidative stress and, hence, protecting β-cells, and increasing the ability for insulin to stimulate glucose uptake in muscles by decreasing their fatigability (Halvatsiotis *et. al.*, 2002), as well as preventing the development of complications due to the production of reactive oxygen and nitrogen species (Evans *et. al.*, 1986).

Various supplements have also been used in the treatment of Type 2 Diabetes with some evidence indicating their effectiveness. For example, calcium (Ghosh & Greenberg, 1995; Bitto *et. al.*, 1997; Hardingham & Badinging, 1999), and chromium (Wilson *et. al.*, 1995; Reviewed in Anderson, 1998a) are required to maintain the production of insulin and its release. Chromium (Vincent, 2000), and magnesium (O'Connell, 2001) appear to play a role in insulin sensitivity and glucose uptake. Vanadium appears to enhance glucose oxidation and glycogen synthesis and it may affect gluconeogenesis (O'Connell, 2001). Lipoic acid (LA) or thiotic acid has been shown to possess anti-oxidant properties (Coleman *et. al.*, 2001) and also to improve skeletal muscle glucose transport (Ramrath *et. al.*, 1999; Maddux *et. al.*, 2001).

Therefore, it may be appropriate to supplement when there are inadequate levels of these nutrients in the diet. There are also many herbs that contain these minerals and, hence, it may be through them that they are effective in decreasing BGLs. Many herbs of the same genus, grown in different areas have varying effects on BGLs and this may be due to the amount of the minerals in the soil.

Even though some herbs used in folk-medicine, or the alternative treatment of Type 2 Diabetes have been the subject of some exploratory studies, while others have been considered more extensively, there is still little reported in credible scientific literature regarding their efficacy, toxicity or mechanisms of action. The aim of this study is to screen a number of popular culinary and traditional folk-medicine herbs, along with some Australian native plants, to determine their ability to decrease BGL. After considering their ability to decrease BGL, one herb, Turmeric, and a combination of Traditional Chinese herbs, Glucostat, were singled out for further study to determine their effect on various murine tissues, such as cardiac muscles, skeletal muscle, and liver. The use of culinary herbs has benefit as toxicity studies will not have to be carried out, because in order to be considered for the study, the herb has already proved itself safe for human use as it has been used in everyday cooking in many cultures throughout the world, for many years. This means a human trial can be carried out more readily, comparing the effects of administration of the herb on BGLs in individuals with prediabetic insulin resistance, to diabetic individuals

not yet on medication (but have consulted with an appropriate medical profession and have been prescribed a low glycemic index diet), and diabetic individuals already taking medications.

# 2. MATERIALS AND METHODS

# 2.1 MATERIALS

Dulbecco's modified Eagle's medium (DMEM) and the antibiotic-antimycotic solution used (10,000 units/ml penicillin G sodium, 10,000 μg/ml streptomycin sulphate, 25 μg/ml amphotercin B, 0.85% saline) were purchased from Gibco, Invitogen Pty Ltd., Mount Waverley, Victoria, Australia, 3149. Standard grade Hamosan Bovine Serum Albumin (BSA) was purchased from Life Sciences, Australia. Wortmannin and Metformin were purchased from Sigma Aldrich Pty Ltd., Castle Hill, NSW, Australia, 1765. Human insulin (Actrapid) was purchased from Novo Nordisk Pharmaceuticals Pty. Ltd., North Rocks, NSW, Australia, 2151. Tissue culture plates and syringe filters (0.45 μm and 0.22 μm) were purchased from Sarstdet Australia Pty Ltd., South Australia, Australia, 5095. All chemicals used were of analytical grade, unless otherwise specified. Glucostat was purchased from Health World Ltd, Eagle Farm, Queensland, Australia, 4009. Herbs were purchased from Austral Herbs, Kentucy, N.S.W., Australia, 2350 and the Armidale Tree Group, Armidale, NSW, Australia, 2350 and Woolworths Supermarket, Armidale, NSW, Australia, 2350. Fresh herbs were taken from the herb garden of the researcher.

# 2.2 PREPERATION OF HERBS

Fifty gm of dried or fresh herb was placed in 1 litre of water and allowed to stand for 24 hours. The extract was filtered through filter paper (Whatman no.1) and the filtrate was stored at -20$^{o}$C until used. Immediately before use, the

solution was sterilized through a 0.22 μmm filter to remove any foreign particles. All herb extractions were prepared at a concentration of 50 gms/L, (equivalent dry weight/volume), all references to concentrations is based on this ratio.

Boiled solutions were prepared by bringing 50 ml of the aqueous extract of the Glucostat and Turmeric to the boil for a period of 10 minutes then being allowed to cool. The solutions were stored at -4$^{o}$C until used. Immediately before use the solution was sterilized through a 0.22 μmm filter to remove any foreign particles.

Turmeric is often used in the cooking of curries, and other cuisines, so 5 gms of the Turmeric was also dry roasted for 5 minutes nefore placing it into the 100 ml water and let stand overnight. This was then stored at -4$^{o}$C until used. Immediately before use the solution was sterilized through a 0.22 μmm filter to remove any foreign particles.

A diethyl ether extraction of each of the aqueous solutions (Turmeric, Boiled Turmeric, Glucostat, Boiled Glucostat) was carried out by placing 200 μl of the individual solutions in each of 3 labelled tubes and adding 1.5 ml diethyl ethanol. Each tube was vortexed for 10 minutes. The tubes were then placed on a bed of dry ice until the aqueous portion was frozen. The supernatant was decanted into steroid assay tubes and placed into the vacuum oven to evaporate

any remaining alcohol. The frozen aqueous portion was reconstituted in 200 µl of distilled water. All material was stored at -18$^{o}$C until used.

# 2.3 ANIMAL ETHICS

All animal experiments using mice were approved by the University of New England Animal Ethics Committee and are in accordance with NH&MRC guidelines for animal experimentations.

# 2.4 *In Vitro* BIOASSAY

## 2.4.1 ANIMALS AND TISSUE PREPARATION

Male Swiss mice aged 40 - 50 days weighing between 18 – 24 gms, obtained from the Physiology mice colony were used in all experiments. The mice were kept in the mouse house at the Physiology breeding house at the University of New England, Armidale, NSW, Australia, 2351, in cages (size 30 cm X 20 cm X 8 cm) with 5 mice per cage. They were given standard rodent chow and water ad lib until experimental time and maintained in a room with temperature set at 21$^{0}$C and light (12:12). They were euthanized by carbon dioxide ($CO_2$) asphyxiation. Their hearts and livers were removed and placed in cold phosphate buffered solution (PBS) of a neutral pH. The hearts amd livers were then cut into 2 ml and 3 ml squares respectively and kept in cold PBS for a maximum period of 2 hours.

### 2.4.2 TISSUE CULTURE ASSAY

An in-house tissue culture method was employed to evaluate the effect of treatments of herbs on tissues involved in glucose homeostasis. Twenty four hour cell culture plates were used and to each well there was 1 ml DMEM supplemented with 0.1% BSA and 1% antibiotic-antimycotic solution, and varying concentrations of glucose (from 5 - 16 mM depending on experiment) and herbal extract added. Normal saline was added to equalize the volume in the wells. The first column was left blank as a control. Then 5-6 pieces of the heart or liver were added to each of the remaining wells, the second column did not contain any treatment. The plate was incubated at 37$^{o}$C in a 5% $CO_2$ environment for 24 hours. The media was then stored at -20$^{o}$C for further analysis.

Wortmananin, insulin (69.45 nmol/L) and Metformin (100 mM) was also added to some of the cultures to determine what biochemical pathways might have been involved and any synergistic/antagonistic effects that may occur.

All the treatments, control and positive control were examined in triplicates unless otherwise specified.

## 2.5 GLUCOSE ANALYSIS

The frozen samples were thawed at room temperature and 100 µl of the media samples was removed and glucose level was determined by the use of the Dade Chemical analyser (DADE-XL, Dupont, USA). The glucose method is an

adaptation of the hexokinase-glucose-6-phosphate dehydrogenase method and is more specific than general reducing sugar methods (Kunst, A., ***et. al.***, 1983).

## 2.6 HUMAN ETHICS

All human trials were approved by the University of New England Human Ethics Committee and are in accordance with NH&MRC guidelines for Human experimentations.

## 2.7 CLINICAL TRIALS

### 2.7.1. Patient Selection.

People who had a fasting blood glucose level of greater then five (>5) as indicated by recent blood tests ordered by their treating Doctors were asked if they would be prepared to participate in the study. The procedure was explained to them and each individual was given the choice of participating or not. There were 11 patients who completed the Turmeric Study and 18 patients who completed the Glucostat study. Patients were asked to continue the diets as prescribed by practitioners.

### 2.7.2. Medical Protocol

The clinical practitioner took a full medical history from patients, along with weights and heights. Previous blood tests results were requested to obtain FBG levels. Pathology Laboratories of the Practitioner and/or patients choice performed these tests. After the determination of the above levels the practitioner discussed with the individual patients their options of treatments.

Each individual was asked to record their FBGLs for 2 weeks using their own hand held monitors or ones donated to the Centre for Bioactive Discovery in Health and Ageing, University of New England, Armidale, NSW, Australia 2351, by Diabetes Australia, NSW Division. The individuals participating in the study were asked to take either 2 capsules of Turmeric (500 mg) twice per day or 1 capsule of Glucostat which contained (*Trichosanthes kirilowii* (1900 mg), *Polygonatum sibiricum* (714.5 mg), *Dioscorea oppostia* (286 mg), *Panax ginseng* (95 mg), and *Stevia rebaudiana* (47.6 mg)) three times per day as per suggested dosage of the supplier. During the trial period of 3 months they were asked to continue taking their FBGLs. At the end of this period people were asked to stop taking either the Turmeric or the Glucostat and were asked to continue taking their FBGLs for a further 3 weeks. At the end of this period those who had responded to treatment were asked to restart the treatment for a further 3 months taking FBGLs daily. All were asked if they would b willing to trial the other herb after the initial 4 month period, with a washout period of 2 months.

## 2.8 DATA ANALYSIS

### 2.8.1. Tissue Culture Results

Basal glucose uptake was calculated based on the difference in glucose concentration between control wells with and without tissues. Glucose uptake in response to various treatments was calculated as a percent variation from basal

levels (% glucose uptake), or percent difference in uptake. All experiments were repeated thrice (n=3) unless specified.

Experimental data were analysed statistically, using the general linear model procedure in SAS statistical software (SAS Institute Inc. Cary, NC, USA). The data was evaluated using 2-way ANOVA followed by Student-Newman Keuls *post hoc* test. Values were considered to be significantly different at $p<0.05$ and presented as mean +/- standard error (+/- S.E.).

# 3. SCREENING OF HERBS FOR DIABETIC EFFECT

# 3. INTRODUCTION

The current orthodox medicines used for the management of Type 2 Diabetes, the oral hyperglycaemic drugs, such as the sulfonylureas, biguanides and thiazolidinediones, appear to be sufficient and BGLs are normalised initially. However, they still tend not to stop the complications found associated with the disease (Moss ***et. al.***, 1994). The continued application of these medications appears to lead to reduced effectiveness, with more of the drug or combination of drugs needing be taken to have the same effect (Moss ***et. al.***, 1994). The use of plants in the treatment of diabetes dates back to approximately 1550 BCE (Gray & Flatt, 1997b). It has been suggested that Aloe Vera was used to treat similar symptoms as would be found in diabetes. In recent years many people are again turning to Herbal and Traditional Therapies as an alternative treatment. More than 400 traditional herbal remedies used for the treatment of diabetes, or similar symptoms, by various cultures have been recorded (See Table 1), however, only a few of these have received medical and scientific evaluation to determine effectiveness, efficacy, side effects and toxicity (Baily and Day, 1989). Since many plants have been used in various cultures for the management of Type 2 Diabetes the World Health Organisation has recommended that medical and scientific examinations of such plants be undertaken (World Health Organisation Expert Committee on Diabetes Mellitus, 1980), to determine their effectiveness, efficacy, side effects and toxicity. There

are also several vitamins and minerals, for example, Vitamins D and E, iron, nicotinamide, that have been examined as far as their role in ameliorating the effects of Type 2 Diabetes (see Table 2), however, little is still known about their efficacy and mechanisms of action.

The ever increasing incidence of Type 2 Diabetes worldwide demands novel solutions for its management. The treatments used today are expensive, and many third world countries are unable to afford them due to low health expenditure, especially with the need for more medications along with combining of medications. Although the current medications have been able to slow the progression of the disease process, none have been able to prevent the progressive loss of insulin production and release, culminating in the need for exogenous insulin therapy. Therefore research into new and novel substances that could preserve the integrity of the pancreatic islet cells, and hence the production of insulin; along with decreasing insulin resistance and enhancement of other peripheral effects, would be beneficial in the advancement of the treatment and management of Type 2 Diabetes.

An aqueous extract of the culinary herbs *Laurus nobilis* tree (Bay Leaves), *Origanum vulgare* (Oregano), *Curcuma longa* Linn (Turmeric), *Ocimum basilicum* (Sweet Basil), *Lavandula officinalis* (English lavender), *Thymus vulgaris* (thyme), *Cuminum cyminum* (Cumin), *Coriandrum sativum* (coriander), *Metha piperita officinalis* (Peppermint), *Myristica fragrans* (Nutmeg),and *Eugenia caryophyllata* (Cloves), along with *Stevia rebaundia,* and *Gymnema*

*sylvestre* and the Australian plants, *Eremophila longofolia* (Emu Bush), Grevilla species leaves and *Stenanethum scortecceni* stems were screened for their ability to increase the uptake of glucose in Swiss mice Heart tissue culture. A combination of Chinese herbs (Glucosat) that is readily available in the Australian market place was also screened for its ability to increase glucose uptake in mouse heart muscle. The screening was important to determine which of the many herbs and plants would be considered for further study at this time.

3. Screening of Herbs for Diabetic Effect

| Herb | Common Name | Model | Type of Extract | Action | References |
|---|---|---|---|---|---|
| *Achyroline satureiodes* | Marcela/maccla | Mice | Whole plant in ethanol | Antidiabetic | Carney et al 2002 |
| *Allium sativum* | Garlic | Animal | | Antidiabetc | Sheela & Augusti, 1992 |
| *Allium cepum* | Opion | Rats | | Antidiabetc<br>Antioxidant. Antidiabetic | Shane-McWhorter, 2001; Bailey & Day, 1989.<br>Kumari & Augusti, 2002 |
| *Arachis hypogaea* | Groundnut or peanut | Rats | Aqueous of nut | Antidiabetic | Bilbis *et. al.*, 2002 |
| *Aronia melanocarpa* | | | Fruit juice | Weight Loss<br>Antidiabetic | Simeonov *et. al.*, 2002 |
| *Artemisia santonicum* | | Rabbits | | Antidiabetic | Korkmaz & Gurdal, 2002 |
| *Barleria lupulina* | | Rats | Ethanol of aerial parts of plant | Antidiabetic, Increased Insulin Secretion | Suba *et. al.*, 2004 |
| *Bauhinia megalandra* | | | Aqueous extract of leaves | Slows Glucose Absorption from gut | Gonzalez-Mujica *et. al.*, 2003 |
| *Bidens pilosa* | | Mice | Ethanol Extract Whole Plant | Antidiabetic | Alarcon-Aguilar *et. al.*, 2002 |
| *Brassica juncea* | Mustard Leaf | Rats | Ethanol | Antioxidant, Antidiabetic | Yokozawa et. al,2002 |
| *Caesalpinia bonducella* | | Rats | | Slows glucose absorption from the gut, deceases gluconeogenesis | Chakrabarti, 2003 |
| *Camellia sinensis* | *Green Tea* | | | Antioxidant | Mustata *et. al.*, 2005 |

Table 1: Herbs and Their Effect on Blood Glucose

| Herb | Common Name | Model | Type of Extract | Action | |
|---|---|---|---|---|---|
| *Cassearia esculenta* | | Rats | Aqueous of Root | Decreased gluconeogenesis, antioxidant, antidiabetic | Prakasam *et. al.*, 2002; Prakasam *et. al.*, 2003a; Prakasam *et. al.*, 2003b |
| *Celosia argentea* | Cocks Comb Cinnamon | Rats | Ethanol of Seeds | Antidiabetic<br><br>Antioxidant, antidiabetic.<br>No improvement in HbA1C or FBGL's | Vetrichelvan *et. al.*, 2003.<br>Anderson *et. al.*, 2004.<br><br>Baker *et. al.*, 2008 |
| *Cissus sicyoides* | Princess Vine | Rats | Leaf decoction | Antidiabetic | Pepato *et. al.*, 2003 |
| *Coccinia indica* | Ivy or Little Gourd | Rats | Ethanol of Leaves | Antidiabetic, Increased Insulin secretion | Pari & Venkataswaran, 2002a |
| *Croton cajucara* | Sacaca | Mice | Bark | Antidoabetic, Anti-inflammatory | Silva *et. al.*, 2001 |
| *Crcuma longa* | Turmeric | Rats | Ethanol of rhizome | Antidiabetic, antioxidant | Arun & Nalini, 2002 |
| *Cyclocarya palliuis* | | Mice | Aqueous of Leaves | Antidiabetic, Improved Insulin secretion, slows glucose absorption in gut | Kurihara *et. al.*, 2003 |
| *Dioscorea opposite* | | Human | | Anecdotal evidence | Bensky & Gamble 1993 |

Table 1 (continued): Herbs and Their Effect on Blood Glucose

| Herb | Common Name | Model | Type of Extract | Action | |
|---|---|---|---|---|---|
| *Eugenia jambolana* | Jamun, Black Plum, Indian Black Berry | Rabbits | Ethanol of Kernels<br><br>Aqueous of Kernels | Antidiabetic, Increased Insulin Secretion.<br>Increased glycolysis, antioxidant | Sharma *et. al.*, 2003<br><br>Ravi *et. al.*,2004 |
| *Ficus carica* | Fig Leaf | Animal | Leaf<br><br>Aqueous and ethanol of leaves | Antidiabetic<br><br>Antioxidant | Campillo *et. al.*, 1991; Torres *et. al.*, 1993; Perez *et. al.*, 1996; Serraelara *et. al.*, 1998.<br>Perez *et. al.*, 2003 |
| *Ficus racemosa* | Gular, Umbar, Jagya-dumbar | Rats | Ethanol of Bark | Antidiabetic | Rao *et. al.*, |
| *Ginko biloba* | | | | No effect | Kudolo *et. al.*, 2002 |
| *Glycyrrhiza uralensis* | | Mice | Ethanol of Bark | PPAR-γ ligand binding activity, antidiabetic | Kurodo *et. al.*, 2003 |
| *Gongronema latifolium* | | Rats | Ethanol of leaves<br><br>Aqueous of leaves | Antioxidant, Antidiabetic, decreased gluconeogensis.<br>Increase hexokinase activity, decrease glucokinase activity, no change in blood glucose | Ugochukwu & Cobourne, 2003.<br><br>Ugochukwu & Babady, 2003. |

Table 1 (continued): Herbs and Their Effect on Blood Glucose

3. Screening of Herbs for Diabetic Effect

| Herb | Common Name | Model | Type of Extract | Action | |
|---|---|---|---|---|---|
| *Gymnema montanum* | | Rats | Aqueous of leaves<br><br>Ethanol of leaves | Increased Insulin secretion, decreased gluconeogenesis.<br>antioxidant | Ananthan *et. al.*, 2003a; Ananthan *et. al.*, 2003b; Ananthan *et. al.*, 2004 |
| *Gymnema sylvestre* | | Rats & Rabbits<br><br>Human | Ethanol of Leaves | Regeneration of islet cells, antidiabetic, increased Insulin secretion.<br>Antidiabetic | Shane-McWhorter, 2001; Persaud *et. al.*, 1999 |
| *Gymnema yunnanense* | | Mice | Ethanol of Leaves | Antidiabetic, Weight loss | Xie *et. al.*, 2003 |
| *Hypoxis hemerocallidea* | African Potato | Rats | Aqueous of tuba | Doe-dependent antidiabetic | Mahomed & Ojewole, 2003 |
| *Inula Britannica* | | Mice | Aqueous of Flowers | Cytoxine production, antidiabetic | Kobayashi *et. al.*, 2002 |
| *Iponomoea batata* | | Animal<br><br>Human | | Increased Insulin sensitivity.<br>Antidiabetic | Ludvik *et. al.*, 2003.<br><br>Ludvik *et. al.*, 2004 |
| *Lyophyllum decastes* | | Mice | Aqueous of fruit | Increase Insulin sensitivity, increase GLUT4 | Miura *et. al.*, 2002 |
| *Momordica charantia* | Bitter Melon | Rabbits<br>Human | | Antidiabetic.<br>Antidiabetic | Akhyas *et. al.*, 1981.<br>Srivastava *et. al.*, 1993; Welhinda *et. al.*, 1986; |

Table 1 (continued): Herbs and Their Effect on Blood Glucose

3. Screening of Herbs for Diabetic Effect

| Herb | Common Name | Model | Type of Extract | Action | Reference |
|---|---|---|---|---|---|
| *Momordica cymbalaria* | | Rats | Aqueous of fruit | Antidiabetic | Kameswararao *et. al.*, 2003 |
| *Morinda officinalis* | | | Aqueous of root<br><br>Ethanol of root<br><br><br>Butanol of root | Dose dependent antidiabetic.<br>Antidiabetic low doses, increased FBGL high doses.<br>Increased FBGL | Soon & Tan 2002<br><br>Soon & Tan 2002<br><br><br>Soon & Tan 2002 |
| *Morus indica* | Mulberry | Rats | Dried Leaf Powder | Antidiabetic | Andallu & Varadacharyulu, 2002 |
| *Mucuna pruriens* | Cowitch | | | Antidiaetic | Dhawan *et. al.*, 1980 |
| *Nigella sativa* | | Rats | Oils of seeds | Regeneration and proliferation of β-cells, increased Insulin secretion | Kanter *et. al.*, 2003 |
| *Ocimum canum* | | Mice | Aqueous fresh and dried leaves | Dose dependent increased insulin secretion | Nyarko *et. al.*, 2002 |
| *Ocinum sanctum* | Holy Basil | Animal | | Antidiabetic | Chattopdhyay, 1993; Agrawal *et. al.*, 1996 |

Table 1 (continued): Herbs and Their Effect on Blood Glucose

| Herb | Common Name | Model | Type of Extract | Action | Reference |
|---|---|---|---|---|---|
| *Panax Quinquefolius* | | Human<br>Human<br><br><br><br>Mice<br><br><br>Mice | <br><br><br><br><br><br><br>Berry | Antidiabetic<br>Increased Insulin secretion, increased number Insulin receptors.<br>Time dependent Increased number Insulin receptors.<br>Increased number Insulin receptors<br>Antidiabetic | Vuskan *et. al.*, 2000.<br>Vuksan *et. al.*, 2001;<br>Sotaniemi *et. al.*, 1995.<br><br><br>Suzuki *et. al.*, 1989.<br><br><br>Dey *et. al.*, 2003 |
| *Phyllanthus sellowianus* | Sarandi blanco | Mice | Aqueous of stem | Antidiabetic, antioxidant | Hnatyszyn *et. al.*, 2002 |
| *Polygonatum sibircum* | | | | Antidiabetic | Bensky & Gamble, 1993 |
| *Psacalium peltatum* | | Mice | Ethanol | Antidiabetic | Alarcon-Aguilar *et. al.*, 2002 |

Table 1 (Continued): Herbs and Their Effect on Blood Glucose Levels

3. Screening of Herbs for Diabetic Effect

| Herb | Common Name | Model | Type of Extract | Action | Reference |
|---|---|---|---|---|---|
| *Pterocarpus marsupium* | Vijaysar | Animals<br>Humans<br><br><br><br><br>Rats | | Antidiabetic<br>Antidiabetic<br>Stimulated Insulin secretion, No effect once diabetic state reached, Regeneration β-islet cells.<br>Inhibits glucose absorption from small intestine, Decrease body weight, Stimulates insulin secretion. | Ahman *et. al.*, 1991a; ICMR, 1998; Charles & Simon, 1984; Hii & Howell, 1984.<br><br><br>ICMR, 1998; Manickam, 1997; Sheenahn *et. al.*, 1982; Chakravarthy *et. al.*, 1982 |
| *Pueraria lobata* | | Rats | Roots | Antidiabetic, Increased GLUT4 | Hsu *et. al.*, 2003 |
| *Ramulus mori* | Sangzhi | Rats & Mice | | Antidiabetic | Ye *et. al.*, 2002a, Ye *et. al.*, 2002b |
| *Salvia officinalis* | | Mice | Aqueous of whole plant | Antidiabetic | Alarcon-Aguilar *et. al.*, 2002 |
| *Sclerocarya birrea* | Marula Tree | Rats | Aqueous of bark | Antidiabetic | Ojewole, 2003 |
| *Scoparia dulcis* | Sweet Broomweed | Rats<br><br>Rats<br><br>Rats | Aqueous of Leaves<br><br>Ethanol & chloroform of leaves<br>Aqueous of leaves | Antidiabetic<br>Antidiabetic, Antioxidant.<br>Stimulates Insulin secretion. | Pari & Venkateswaran, 2002b.<br>Latha & Pari, 2003b.<br>Latha et. al,2004 |
| *Scrophularia deserti* | Afinah, Zetab, Jar, Maseelah | | | Antidiabetic, Anti-inflammatory | Ahmed *et. al.*, 2003 |

Table 1 (Continued): Herbs and Their Effect on Blood Glucose Levels

3. Screening of Herbs for Diabetic Effect

| Herb | Common Name | Model | Type of Extract | Action | Reference |
|---|---|---|---|---|---|
| *Sesamum indicum* | Sesame Seeds | | Aqueous from seeds | Antidiabetic, decreased glucose absorption from gut | Takeuchi *et. al.*, 2001. |
| *Silibum marianum* | Milk Thistle | | | Antidiabetic, Antioxidant | Velussi *et. al.*, 1997; Shane-McWhorter, 2001 |
| *Stephania tetrandra* | | Mice | | Antidiabetic | Tsutsumi *et. al.*, 2003 |
| *Stevia rebaudiana* | | Human<br>Rats<br><br><br>Rats | | Antidiabetic.<br>Increased Insulin secretion, Increased Hepatic Glycogen synthesis.<br>Antidiabetic, Insulintropic, glucagonostatic | Curie 1986.<br>Malaisse *et. al.*, 1998.<br><br><br>Jeppesen *et. al.*, 2000 |
| *Tecoma stans*<br>(Plants Mexico)<br>(Plants Brazil & Egypt) | | Rabbits<br>Rabbits | | Antidiabetic<br>No effect | Costantino *et. al.*, 2003. |
| *Terminalia pallid* | | Rats | Fruit | Antidiabetic | Kameswara *et. al.*, 2003 |
| *Trichosanthes kirilowii* | | Mice | Whole Plant | Antidiabetic<br>Anti-inflammatory | Hikino *et. al.*, 1989.<br>Xu *et. al.*, 2001 |

Table 1 (Continued): Herbs and Their Effect on Blood Glucose Levels

| Herb | Common Name | Model | Type of Extract | Action | Reference |
|---|---|---|---|---|---|
| *Trigonella foenum graecum* | Fenugreek | Skeletal Muscle<br><br>Liver | Leaves<br><br>Leaves | Antidiabetic, Decreased gluconeogenesis.<br>Decreased gluconeogenesis, increased glycolysis | Vats *et. al.*, 2003.<br><br>Devi *et. al.*, 2003 |
| *Tuernera diffusa* | | Mice | Aqueous of leaves | No effect | Alarcon-Aguilar *et. al.*, 2002 |
| *Zingiber officinale* | Ginger | Mice & Rats | | Antidiabetic | Akhani *et. al.*, 2004 |

Table 1 (Continued): Herbs and Their Effect on Blood Glucose Levels

| Vitamin/Mineral | Model | Action | Reference |
|---|---|---|---|
| Calcium | Human | Chronically high levels lead to glucose unresponsiveness. Normalisation will increase expression of $K_{ATP}$ channels and voltage dependent Calcium channels. Intakes inversely associated with Type 2 diabetes risk. | Kohtaro *et. al.*, 2002. Pittas *et. al.*, 2006. Shahar *et. al.*, 2007. |
| | Human | Decreases Insulin resistance in the normal and IFG patient. Weight Loss with dairy calcium | |
| Carnitione | Mouse | Decreases lipid and glucose intolerance. Enhances mitochondrial efflux. | Power *et. al.*, 2007 |
| Chromium | Humans/ Animal | Potentates' insulin action. Enhanced glucose uptake in skeletal muscle cells. Increased insulin receptors. Affects β-cell sensitivity. Inhibits tyrosine phospatase, an enzyme that dephosphorylates the post-receptor insulin molecule thereby reducing insulin action. Increased insulin sensitivity. Some say no effect. | Reviewed Mertz *et. al.*, 1998; Vincent, 2000; Anderson, 2000; Anderson *et. al.*, 1987; Anderson *et. al.*, 1997; Goldstein, 2000. |
| | Humans | No effect on non-diabetic subjects. | Althius *et. al.*, 2002; Reviewed Lukaski, 1999; Reviewed in Hellerstein, 1998. |
| | Humans | Chromium picinolate – decreased BGL's and circulating insulin levels. Enhanced by nicotinic acid. | Althius *et. al.*, 2002. Anderson *et. al.*, 1997. |
| | Rats | Increased insulin sensitivity. Preservation of renal function. | Urberg, 1987. Mozaffari *et. al.*, 2005 |

Table 2: Vitamins and Minerals and effect on Blood Glucose Levels

| Vitamin/Mineral | Model | Action | Reference |
|---|---|---|---|
| Ferritin | Humans | Increased ferritin levels increased risk of type 2 Diabetes independently of other risk factors; Inverse correlation with adiponectin levels; iron deposition in liver may suppress hepatic glucose response. | Forouhi *et. al.*, 2007. |
| Magnesium | <br><br><br><br><br>Humans<br><br>Humans | Insulin resistance – mechanism unknown, possibly due to co-factor in various enzymatic pathways.<br>Increased insulin secretion.<br>Improved insulin sensitivity.<br>No effect on gluconeogenesis.<br>Insulin resistance due to low magnesium concentrations.<br>Improved insulin resistance. | O'Connell, 2001.<br>Mooradian *et. al.*, 1994.<br><br><br>Meyer *et. al.*, 2000.<br>Paolisso *et. al.*, 1992 |
| Selenium | Humans | Positively associated with diabetes. | Bleys *et. al.*, 2007. |
| Vanadium | <br>Humans<br><br><br>Rats<br><br><br>Human | Enhance glucose oxidation and glycogen synthesis. Decrease gluconeogenesis.<br>Decreased FBGL's, Decreased HbA1c levels.<br><br><br>Decreased BGL's, Increased insulin release, GLUT4 translocation.<br>Severe side effects –given with black Lichee Tea – no side effects and potent hypoglycaemic effect both acutely and chronically.<br>Antioxidant. Appears safe as supplement | O'Connell, 2001.<br>Boden *et. al.*, 1996;<br>Halberstrom *et. al.*, 1996;<br>Cohen *et. al.*, 1995.<br>Clarke *et. al.*, 2004.<br><br><br>Foster 2007. |

Table 2 (Continued): Vitamins and Minerals and effect on Blood Glucose Levels

| Vitamin/Mineral | Model | Action | Reference |
|---|---|---|---|
| Vitamin D | Human | Intake inversely associated with Type 2 Diabetes risk. Increase insulin sensitivity, increased insulin secretion, Vitamin D receptors on β-cells. | Pittas *et. al.*, 2006. |
| Carotenoids | Human | Inversely associated with Diabetes<br>Antioxidants | Coyne *et. al.*, 2005 |

## 3.2. MATERIALS and METHODS

Plant extraction, ethics approval, tissue preparation, and tissue culture assay; glucose analysis; statistical analysis have been described in Chapter 2 of this thesis. Each herb was tested in duplicate for this screening.

*Curcuma longa* Linn (Turmeric) was divided into three groups. One was purchased as a dry powder from Woolworths in Armidale, NSW, Australia 2350; the second, a dry powder and the third, Turmeric cuts, both purchased from Austral herbs, Kentucky, NSW, Australia, 2350

## 3.3. RESULTS

An aqueous extract of the leaves from the *Laurus nobilis* tree (Bay Leaves) at the concentration of 10 μg/ml showed an inhibitory effect on the uptake of glucose by the heart tissue culture with a decrease of 5.4 +/- 1.2% uptake over the heart tissue itself. The aqueous solution showed a little difference in percent uptake with 2.28 +/- 0.97% being noted for the 5 μg/ml solution. The 2.5 μg/ml solution resulted in an increase of 4.13 +/- 1.3%. However, the concentration of 1.25 μg/ml showed an increase uptake of 5.89 +/- 1.54%. As the lower concentrations elucidated a better uptake, a decrease in concentration was tested with 1 μg/ml of the Bay Leaf resulting in a 6.75 +/- 0.54% uptake; and at a concentration of 0.5 μg/ml the percent in uptake was 4.74 +/- 0.26%; and at a concentration of 0.25 μg/ml it was 2.68 +/- 0.34% (See Figure 3.1B).

Concentrations of 10, 5, 2.5 and 1.25 μg/ml of aqueous extract of *Origanum vulgare* (Oregano), were also examined in mice heart tissue culture where it was noted that all concentrations had a slight inhibitory effect. The 10 μg/ml concentration of Oregano showed an inhibitory effect of -3.48 +/- 0.895%; while the 5 μg/ml showed an inhibitory effect of -4.77 +/- 1.2%. The inhibitory effect for the Oregano concentration of 2.5 μg/ml was -2.68 +/- 0.32% while that of the 1.25 μg/ml was -1.36 +/- 0.54% (See Figure 3.1B).

The Woolworths' purchased Turmeric showed an increase uptake at all concentrations. At a concentration of 10 μg/ml the Woolworths' Turmeric showed an increased glucose uptake in the mice heart tissue culture of 12.9 +/- 0.85%; while that of a concentration of 5 μg/ml showed an increase uptake of 15.9 +/- 3.7%. At a concentration 2.5 μg/ml there was an increased uptake of 13.3 +/- 2.0% while at 1.25 μg/ml it was 5.5 +/- 1.45%. The Austral Herbs dried Turmeric powder and the Turmeric cuts, which had been ground down, showed the same results. At all the concentrations there was an increase in the glucose uptake of the mice heart tissue culture. However if the Turmeric has been dry roasted the results are significantly different with results of 2.93 +/- 0.2% at a concentration of 10 μg/ml, 4.44+/- 0.32% at a concentration of 5μg/ml, 1.2 +/- 0.7%; at a concentration of 2.5 μg/ml and 0.47 +/- 0.34% at a concentration at 1.25 μg/ml (See Figure 3.1A).

Fresh *Ocimum basilicum* (sweet basil) increased the glucose uptake of the mouse heart tissue by 9.96 +/- 0.78%, 10.67 +/- 0.45%, 11.95 +/- 0.34% and 10.53 +/- 0.43% when concentrations of 10 μg/ml, 5 μg/ml, 2.5 μg/ml and 1.25 μg/ml were used respectively; however, dried basil exhibited an increase of only 1.2 +/- 0.24%, 1.7 +/- 0.56%, 1.9 +/- 0.37% and 1.5 +/- 0.67% respectively at these same concentrations (See Figure 3.1A).

Fresh *Lavandula officinalis* (English lavender) increased the glucose uptake of the mouse heart tissue by 7.55 +/- 0.54% at a concentration of 10 μg/ml, 7.27 +/- 0.87% at a concentration of 5 μg/ml, 7.46 +/- 0.38% at a concentration of 2.5 μg/ml and 7.55 +/- 0.26% at a concentration of 1.25 μg/ml (see Figure 3.1B).

Dried *Thymus vulgaris* (thyme) had a decrease of 0.62 +/- 0.34% at the highest concentration of 10 μg/ml; and then exhibited an increase of 4.7 +/- 0.38%, 7.26 +/- 0.64% and 9.53 +/- 0.12%, as the concentrations decreased from 5 μg/ml, 2.5 μg/ml and 1.25 μg/ml respectively. Due to the increased glucose uptake at lower concentrations, further studies were considered at even lower concentrations, and at a concentration of 1 μg/ml of the thyme there was a 9.31 +/- 0.26% uptake; and at a concentration of 0.5 μg/ml the percent uptake was 9.28 +/- 0.53%; and at a concentration of 0.25 μg/ml it was 7.97 +/- 0.46 % (See Figure 3.1A).

*Cuminum cyminum* (Cumin) at concentrations of 10 μg/ml, 5 μg/ml, 2.5 μg/ml and 1.25 μg/ml resulted in an increased up take of glucose by the mouse heart tissue by 1.68 +/- 0.38%, 1.8 +/- 0.54%, 0.56 +/- 0.17% and 0.3 +/- 0.78% respectively (See Figure 3.1B).

Dried *Coriandrum sativum* (coriander) decreased glucose uptake by -6.69 +/- 1.4% at a concentration of 10 μg/ml; however, at lower concentrations, there was little change in glucose uptake from the control, with a percentage uptake of 2.83 +/- 0.34%, 0.45 +/- 0.64%, and 0.11 +/- 0.48% at the concentrations of 5 μg/ml, 2.5 μg/ml and 1.25 μg/ml respectively (See Figure 3.1B).

Fresh *Metha piperita officinalis* (Peppermint) showed little change in glucose uptake by the heart mouse tissue, with a percentage uptake of 0.94 +/- 0.2%, 0.91 +/- 0.17%, 0.84 +/- 0.26%, and 0.94 +/- 0.19% at the concentrations of 10 μg/ml, 5 μg/ml, 2.5 μg/ml and 1.25 μg/ml respectively (See Figure 3.1B).

Ground *Myristica fragrans* (Nutmeg) consistently showed an increase uptake of glucose by the mouse heart tissue, of 4.31 +/- 0.28%, 4.11 +/- .46%, 4.13 +/- 0.2% and 4.69 +/- 0.9% at the concentrations of 10 μg/ml, 5 μg/ml, 2.5 μg/ml and 1.25 μg/ml respectively (See figure 3.1B).

Ground *Eugenia caryophyllata* (Cloves) exhibited a slight increased uptake of glucose by the mouse heart tissue ,with a percent increase of 3.69 +/- 0.43%, 3.9 +/- 0.76%, 2.02 +/- 0.54% and 1.58 +/- 0.47% over the ranges of

concentrations of 10 μg/ml, 5 μg/ml, 2.5 μg/ml and 1.25 μg/ml respectively (See Figure 3.1A).

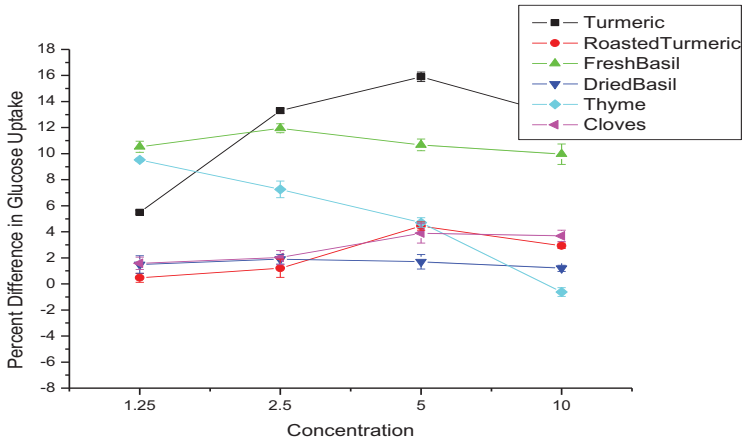
*Stevia rebaundia* exhibited a percent increase of glucose uptake by the mouse heart tissue, of 9.81 +/- 0.14%, 9.24 +/- 0.56%, 8.88 +/- 0.48% and 8.25 +/- 0.37% at the concentrations of 10 μg/ml, 5 μg/ml, 2.5 μg/ml and 1.25 μg/ml respectively (See Figure 3.1C).

*Gymnema sylvestre* resulted in a percent increase of glucose uptake by the mouse heart tissue, of 12.5 +/- 0.63%, 12.6 +/- 0.75%, 9.87 +/- 0.26%, and 5.6 +/- 0.39% over the concentration range of 10 μg/ml, 5 μg/ml, 2.5 μg/ml and 1.25 μg/ml respectively (See Figure 3.1C).

The Australian plants showed a wide range of results with *Eremophila longofolia* (Emu Bush) resulting in an increased glucose uptake by the mouse heart tissue of 12.3 +/- 0.44%, 13.7 +/- 0.61%, 10.58 +/- 0.39%,and 7.45+/- 1.2% respectively, at the concentrations of 10 μg/ml, 5 μg/ml, 2.5 μg/ml and 1.25 μg/ml (See Figure 3.1C). Grevilla species leaves at lower concentrations, exhibited a slight increase of glucose uptake in the heart tissue with a 6.78 +/- 0.98% increase at the concentration of 1.25 μg/ml; however, at concentrations of 10 μg/ml, 5 μg/ml, and 2.5 μg/ml respectively, there was a decrease in uptake of -6.48+/- 1.5%, -3.41 +/- 0.87%, and -2.78 +/- 0.75% (See Figure 3.1C). *Stenanethum scortecceni* stems showed little difference in the glucose uptake by

3. Screening of Herbs for Diabetic Effect

A

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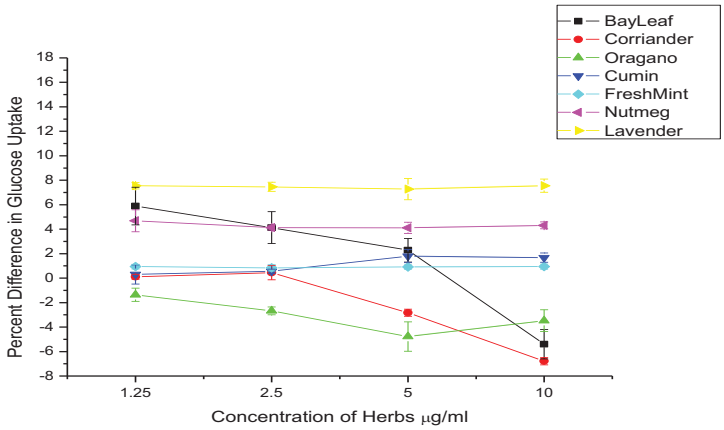



C

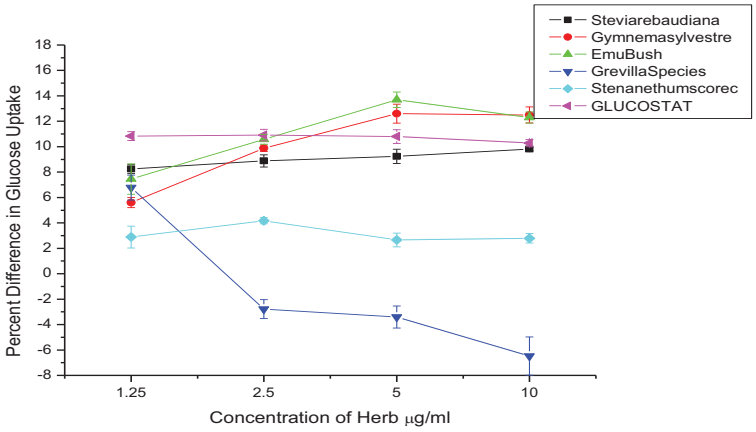



Figure 3.1: The difference between basal glucose uptake in Swiss mice heart tissue and the uptake at various concentrations of different herbs.

the heart tissue over the control at all concentrations, with a percentage difference of 2.79 +/- 0.37%, 2.66 +/- 0.54%, 4.17 +/- 0.24% and 2.89 +/- 0.86% (See Figure 3.1C).

Glucostat, which is a combination of the Chinese herbs including *Trichosanthes kirilowii* (1900 mg), *Polygonatum sibiricum* (714.5 mg), *Dioscorea oppostia* (286 mg), *Panax ginseng* (95 mg), and *Stevia rebaudiona* (47.6 mg) per capsule, was also screened for its effectiveness in decreasing glucose levels by the mouse heart tissue. It showed an increase of glucose uptake by the mouse heart tissue of 10.28 +/- 0.28%, 10.8 +/- 0.54%, 10.91 +/- 0.46% and 10.83 +/- 0.35% at the concentrations of 10 μg/ml, 5 μg/ml, 2.5 μg/ml and 1.25 μg/ml respectively (See Figure 3.1C).

Due to the increased uptake of glucose by the mouse heart tissue at the lower concentrations of the leaves from the *Laurus nobilis* tree (Bay Leaves) a decreased concentration was tested with 1 μg/ml of the Bay Leaf, resulting in a 6.75 +/- 0.54% uptake and at a concentration of 0.5 μg/ml the percent in uptake was 4.74 +/- 0.26% and at a concentration of 0.25 μg/ml it was 2.68 +/- 0.34% (See Figure 3.2).

Due to the increased glucose uptake at lower concentrations of the dried *Thymus vulgaris* (thyme), further studies were considered at lower concentrations, and at a concentration of 1 μg/ml of the thyme there was a 9.31 +/- 0.26% uptake, and at a concentration of 0.5 μg/ml the percent uptake was

9.28 +/- 0.53%, and at a concentration of 0.25 μg/ml it was 7.97 +/- 0.46 % (See Figure 3.2).

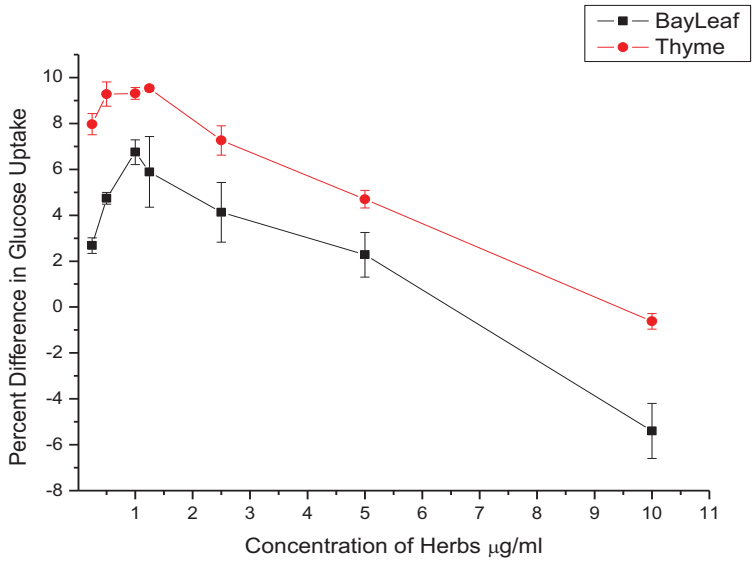



Figure 3.2: The difference between basal glucose uptake in Swiss Mice heart tissue and the uptake at various concentrations of different herbs.

## 3.4. DISCUSSION

The increasing prevalence of Type 2 Diabetes requires the development of novel treatments, and plants have been the source of medications from ancient times, and many of our modern day western medications have been derived from plants, for example Metformin. Many throughout the world recognise the use of native plants for the treatment of many diseases and disorders, including diabetes. However, little if any scientific study has been carried out to support these claims. The World Health Organisation has recommended that medical and scientific examinations of such plants be undertaken (World Health

Organisation Expert Committee on Diabetes Mellitus, 1980), to identify their effectiveness in the management of Type 2 Diabetes, in an attempt to use affordable means for all countries to meet the need of the people, where there is an ever increasing number of sufferers.

Culinary herbs are used widely around the world to flavour and colour food; however, little is known concerning the effect that they might be having on health. It is assumed that they are safe for consumption at the levels that they are used in cooking, or in fresh form as no serious side effects or death has resulted from them, unless an allergic reaction has occurred; however, this appears to occur on only very rare occasions. Many of these herbs may be advantageous to health; however, little if any scientific evidence has been forthcoming on the efficacy, efficiency, toxicity; the best method of administration, the active constituents, and the biochemical pathways/systems involved of these herbs, to obtain medicinal effects. The effects of preparation of the herbs may also have and effect on their medicinal value.

A preliminary study, looking at the effects of some of the culinary herbs on the glucose uptake in Swiss male mice heart tissue, was undertaken. The reason for this was to determine which herb/s warranted further study, to determine if they would be of any benefit in the management of Type 2 Diabetes, by decreasing high FBLs. The use of culinary herbs in the diet would be beneficial, especially in third world countries as the cost could be kept to a minimum. Of the herbs tested a few have had some preliminary research undertaken to

determine effectiveness as far as diabetes treatment however, they have generally been with ethanolic extracts where as aqueous extracts have been used in this study and so may be looking at different active ingredients. Some had not been tested as far as can be determined from a literature search.

The preparation of herbs and tissue culture protocol used through out the study, was developed based on a number of experiments (data not shown), focusing on the viability of the heart tissue through the incubation period. The basal glucose uptake by muscle has been reported as one of the most sensitive indicators of *in vitro* functional viability (Dohm *et. al.*, 1988); and therefore in this study, basal glucose uptake was considered as the indictor for metabolic viability.

An aqueous extract of the leaves from the *Laurus nobilis* tree (Bay Leaves) at higher concentrations, showed an inhibitory effect on the glucose uptake; however, as the concentration was decreased there was an increase in glucose uptake, with the maximum being 6.75 +/- 0.54% at a concentration of 1 μg/ml. At lower concentrations the uptake began to decrease again. The Bay Leaf may have been toxic in high concentrations, and this may have been why the inhibitory effect was shown initially. As the concentration decreased the toxicity also decreased allowing an active ingredient to increase the glucose uptake in the Swiss mice heart tissue culture. Cinnamtannin B-1, found in Bay Leaves, has been shown to have any antioxidant effect (Ferreira, *et. al.*, 2006; Bouaziz, *et. al.*, 2007). This may have resulted in the increased uptake of glucose once the

toxicity had been diluted out. Fetrow and Avila (2001) showed that Bay Leaf will increase the action of Insulin. However, as this is a crude extract there might also be toxic effects present at higher concentrations.

The aqueous extract of *Origanum vulgare* (Oregano) resulted in an inhibitory effect on the uptake of glucose by the Swiss mice heart tissue. In a study carried out by Lemhadari and colleagues (2004), they showed that an aqueous extract of oregano decreased BGLs in streptozotocin (STZ) rats, after both a single dose, and also after 15 daily doses. It was also noted that basal insulin levels were not affected. The difference in our results can be explained by the fact that we were using crude extract *in vitro.* Some toxic effects may have resulted, whereas the previous study was *in vivo,* and some of the toxic compounds may have been removed in the digestive process of the rats.

*Curcuma longa* Linn, more commonly known as Turmeric, is used widely throughout the world as a flavouring and colouring in cooking. The main colouring agent of Turmeric is curcumin (diferuloylmethane), and it has been shown to have a wide range of effects including antihepatotoxic, antiinflammatory, hypocholesterolemic, choleretic and hydrocholagocic, hypoglycaemic. It also brings about an increase in gastric mucosal secretion in rabbits (Arun & Nalini, 2002).

*Curcuma longa* Linn (Turmeric) was divided into three groups. One was purchased as a dry powder from Woolworths in Armidale, NSW, Australia, 2350; the second, a dry powder and the third, Turmeric cuts, both purchased

from Austral Herbs, Kentucky, NSW, Australia, 2350. This was done to determine if there would be in difference in where it was purchased from and in what state of processing. The results were all within the same range, so it appears that the purchase place and the degree of grinding of the rhizome does not make any difference, to the effect that the Turmeric has on the Swiss Mice heart tissue. In all three groups and at all concentrations, there was an increase in the glucose uptake of the Swiss mice heart tissue culture, with the greatest uptake being at a concentration of 5 μg/ml. There was then a slight decrease in glucose uptake as the concentration increased, and this may be due to the fact that a crude extract was being used; hence a toxicity factor entered into the equation. As Turmeric is used for making curry powder and curries of various types, it was decided to test the effects of dry roasting the powder on the ability to increase glucose uptake by the Swiss mice heart tissue. The Turmeric powder was dry roasted in a pan over high heat for 3 minutes, as would be done in the cooking process. This resulted in no change of glucose uptake over basal levels. It can be concluded from this that the dry roasting deactivates the active constituent within the Turmeric, and implies that the eating of curries in the diet will not assist in the increase of glucose.

Fresh *Ocimum basilicum* (Sweet Basil) showed a moderate increase in the glucose uptake of the mouse heart tissue over all concentrations; however, dried basil exhibited a negligible increase, suggesting that the drying has inactivated the active constituents of the herb. The activity of the fresh basil may be due to

its antioxidant affect, as shown by Gulcin and colleagues (2007); also its anti-inflammatory affect as shown by Singh (1999).

Fresh *Lavandula officinalis* (English lavender) increased the glucose uptake of the mouse heart tissue by approximately 7.55% +/-0.54% at all concentrations tested. Dried English Lavender was not tested at the time, to determine if this would decrease the uptake, due to its non-availability. Two studies have identified antioxidant effects of aqueous extracts of different species of Lavender, and this may account for the mechanism of how this plant is decreasing BGls (Hohmann *et. al.*, 1999; Nitzsche *et. al.*, 2004).

Dried *Thymus vulgaris* (thyme) had a decrease of glucose uptake as the concentration decreased. Therefore a further study was considered at lower concentrations. As the concentration decreased there was an increase of glucose uptake until a dilution of 0.5 µg/ml was reached, when there was a slight fall in the glucose uptake. These results may be indicative of the antioxidant effect of the thyme as indicated by Kulisic and colleagues (2007), which showed that both the essential oils and aqueous tea infusions of thyme, exhibited an antioxidant effect. This may due to the large amount of polyphenols contained in the plant. Vigo and colleagues (2004) noted an anti-inflammatory action of thyme. This may have also played a role in the increase of the glucose uptake. It was noted that the effect appeared to increase at lower concentration of the thyme. This may be due to a decrease in a toxic component and purification of the active compound may yield better results. Further studies would need to be

carried out on this extract, to determine the active ingredient/s, and to look at its toxicity.

*Cuminum cyminum* (Cumin), at all concentrations, resulted in only a small increase in the glucose uptake in mouse heart tissue. Cuminaldehyde, an aldose reductase and alpha-glucosidase, found in the seeds of cumin has been shown to exhibit some effect on glucose levels, but 1.6 – 1.8 percent less effective than that of quercetin and acarbose (Lee, 2005). However, as the use of an aqueous solution did not select for this compound, it seems unlikely that this would have resulted in the slight increase that was found. Roman-Ramos and colleagues (1995) showed that Cumin acted as an antidiabetic agent in rabbits. Dhandapani and colleagues (2002) work supported this, and showed that Cumin was more effective then glibenclamide in the management of Type 2 Diabetes. They also showed that it exhibited a hypolipidemic effect in rats. This difference may be due to the current experiments looking at the uptake in mouse heart tissue, rather then in alloxan treated diabetic rats. Together with many other herbs, cumin also exhibits antioxidant effects (Satanarayana *et. al.*, 2004). This may have lead to the increased glucose uptake that was shown in this experiment.

Dried *Coriandrum sativum* (coriander) decreased glucose uptake at higher concentrations, in the mouse heart tissue, suggesting toxicity at these levels; however, at lower concentrations there was a slight increase in uptake. This may be accounted for by the anti-oxidant effect as described by Satyanarayana and colleagues (2004) and Apak and colleagues (2006). Gray and Flatt (1999)

showed that whole coriander exhibited an insulin like activity, and insulin releasing activity in streptozotocin-induced diabetic mice, as well as increased glucose uptake and conversion to glycogen. This does not correspond with the results obtained at this time, which may be due to the crude leaf extract that was used in current experiment.

Fresh *Metha piperita officinalis* (Peppermint) showed little change in glucose uptake by the heart mouse tissue, possibly due to the antioxidant capacity found in most fresh herbs, although no literature could be found that to support this.

Ground *Myristica fragrans* (Nutmeg) consistently showed an increase of glucose by the mouse heart tissue of approximately 4.5% at all concentrations. Yang and colleagues (2006) showed that lignans from nutmeg inhibited Protein Tyrosine Phosphatase 1B. This may enhance the intracellular insulin signalling, which may account for the consistent increase that was seen. However, further investigation may determine if this is the mechanism of action.

Ground *Eugenia caryophyllata* (Cloves) exhibited a slight increased uptake of glucose uptake by the mouse heart tissue, which may be a result of its antioxidant capacity (Yadav & Bhatnagar, 2007). Broadhurst and colleagues (2000) have suggested that cloves exhibited an increase in insulin activity, but this was removed with the treatment of poly vinylpyrrolidone suggesting that the active constituent is a phenol, which could also a play a role in the results obtained in this assay.

*Stevia rebaundia* exhibited a 9.24 +/- 0.56% increase of glucose uptake by the mouse heart tissue, at the concentrations of 5 μg/ml; with a greater increase at higher concentrations, and a slight decline as concentrations decreased. The *Stevia rebaudiana* plant and its constituents have had a controversial history as far as its antidiabetic effects are concerned (White 1994). Curie (1986) showed a decreased BGL in humans after administration of 20 gm/day, and postulated that it was due to an increase in the mitochondrial respiration rate or inhibition of gluconeogenesis. It has also been shown to exert an antihyperglycaemic, insulinotropic and glucagonostatic effect in the Type 2 Diabetic GK rat. It is thought that this might be due to a direct action on β-cells (Jeppesen *et. al.*, 2000). Experiments performed by Malaisse and colleagues (1998) showed that in the presence of stevioside, there was an increase of insulin output. However, it only reached a statistically significant effect at the higher concentrations. It has also been shown to act as a stimulant on hepatic glycogen synthesis, under gluconeogenic conditions in rats. The current assay indicates that it also has a direct action on the muscle cell, and increases the glucose uptake. Although without further investigations the mechanism of action cannot be determined.

*Gymnema sylvestre* resulted in an increase of glucose uptake by the mouse heart tissue of 12.5 +/- 0.63%, at a concentration of 10 μg/ml. This declined as the concentrations decreased. *Gymnema sylvestre* has been shown to exhibit effects on the β-cells, resulting in increased insulin secretion (Sugihara *et. al.*,

2000). However, this would not have resulted in the increase in glucose in current assay. Tominaga and colleagues (1995) looked at the effect of *Gymnema sylvestre* on insulin resistance and showed that it was improved; however, no mechanism of action was elucidated from the results obtained. Whilst Shanmugasundaram and colleagues (1983) showed that it resulted in an increase in the activity of the enzymes involved in the insulin-dependent pathway, which resulted in an increased uptake of glucose.

The Australian plants showed a wide range of results, with *Eremophila longofolia* (Emu Bush) resulting in an increased glucose uptake by the mouse heart tissue. Whilst the Grevilla species leaves at lower concentrations exhibited a slight increase of glucose uptake; however, at lower concentrations there appeared to be a toxic affect with a decrease in glucose uptake. *Stenanethum scortecceni* stems showed little difference in the glucose uptake by the heart tissue over the control at all concentrations. Only a small amount of research has been conducted on Emu Bush, and is continueing in this laboratory to determine its effectiveness as an antidiabetic. The other plants have never been considered as a management proposition for diabetes and were included in the screening process to determine if further investigation should be undertaken at this time.

Glucostat, which is a combination of the Chinese herbs including *Trichosanthes kirilowii* (1900 mg), *Polygonatum sibiricum* (714.5 mg), *Dioscorea oppostia* (286 mg), *Panax ginseng* (95 mg), and *Stevia rebaudiona*

(47.6 mg) per capsule, was also screened for its effectiveness in decreasing glucose levels by the mouse heart tissue. It showed a moderate (approximately 10% over basal) percentage increase of glucose uptake by the mouse heart tissue. Some research has been carried out on the individual herbs, but this is the first time that any research has been carried out on the combination, as far as can be traced. An exception would be an introductory clinical trial undertaken as an Honours project (Collins and McFarlane, 2006). When looking at combinations of herbs, both synergistic and antagonistic effects need to be taken into consideration. Simply because they are effective individually they may not be in combination. Of the herbs contained in Glucostat, more work has been carried out on *Trichosanthes kirilowii*, *Panax ginseng* and *Stevia rebaudion.* Little research has been carried out on *Dioscorea oppostia,* while *Polygonatum sibiricum* had only had anecdotal evidence as to their effectiveness in the management of Type 2 Diabetes.

Shan yao from the *Dioscorea opposita* plant has also been used for centuries in Traditional Chinese Medicine. It is also the principle herb in a combination of Chinese herbs (Die-Huang-Wan), which showed increased insulin sensitivity in rats (Hsu, J., *et. al.*, 2007).

Panex ginseng was shown by Vuksan and collegues (2001) and Sotaniemi and colleagues (1995) to decrease fasting BGL in both diabetic and non-diabetic patients. The studies showed an enhancement of insulin release from the pancreas, and an increase in the number of insulin receptors. This is as a

possible mechanism of action for the current assay. Suzuki (1989) supported this by showing in increase in the number of insulin receptors and a resulting reduction in insulin resistance. Sotaniemi and colleagues (1995) suggested that an unspecified species of ginseng may be useful in the management of Type 2 Diabetes. This may be due to direct action where there was improved insulin sensitivity, and increased glycogen storage, independent of diet and physical activity.

Huangjing or Siberian Solomon's Seal is from the *Polygonatum sibiricum* plant. This herb has been used in Traditional Chinese Medicine for centuries for the treatment of wasting and thirsting disease. However, little research has been conducted on its effectiveness (Bensky & Gamble 1993); with one study showing that after an initial rise in glucose, the herb decreases blood sugar levels and can inhibit ephedrine-induced hyperglycaemia.

The *Stevia rebaudiana* plant and its constituents have had a controversial history as far as its antidiabetic effects are concerned (White 1994). Curie (1986) showed a decreased BGL after administration of 20 gm/day, and postulated that it was due to an increase in the mitochondrial respiration rate or inhibition of gluconeogenesis. It was shown to exert an antihyperglycaemic, insulinotropic and glucagonostatic effect in the Type 2 Diabetic GK rat, possibly due to a direct action on β-cells (Jeppesen *et. al.*, 2000).

Gua lou, from the root tubers of the *Trichosanthes kirilowii* plant, has been used in Chinese medicine for the 'Thirsting Disease' for centuries. Hikino and

colleagues (1989) found that the non-dialyzable portion decreased BGL in mice. They isolated five glycans (Trichosans A, B, C, D, and E), all of which showed hypoglycaemic actions in normal mice. Trichosan A also had this effect in alloxan-induced hyperglycaemic mice. Xu and colleagues (2001) have shown that trichosantinius also has anti-inflammatory properties, and this may be the process that aids in the management of Type 2 Diabetes, as it has now been shown that inflammation and Type 2 Diabetes are linked.

Many herbs that we use in our kitchens everyday may be beneficial in maintaining or decreasing our BGLs; however, there seems to be a trend away today from cooking at home and using these herbs rather relying on fast foods that contain a lot of fat for flavour. The use of fresh and dried herbs to flavour our foods may be part of the answer to the diabetic epidemic that is occurring in the world today, along with the taking of herbs as teas or in other forms, in order to keep blood glucose levels within the normal range. The results of research thus far conducted in this and other laboratories indicates that there are many herbs which may be of benefit in maintaining a healthy FBGL range and in allowing our bodies to function more effectively and efficiently. Further research needs to be conducted on all the culinary herbs and many plants around in the world, to determine their effectiveness, and possible use in the management of Type 2 Diabetes.

It was determined to conduct further research on Turmeric, as it is a readily available and cost effective herb which appears to be safe, with no noted major

side effects if taken properly. If it does result in being effective in decreasing BGLs, it would be a cost effective way to treat the surge in diabetes in the third world countries, where cost of production is often a major factor in any medications. People could cultivate the herb for themselves in many areas of the world. It was also deyermined to look further at Glucostat; a combination of Chinese herbs available in Australia and often prescribed by Natural Therapists/Herbalists for the management of Type 2 Diabetes. To date no known research has been carried out to its effectiveness as a combination, even though some of the individual herbs have had some research carried out on them. As Glucostat is commercially available, it should also not present a safety issue, when taken according to instructions. There by not preventing any human trials being undertaken.

# 4. EFFECT OF TURMERIC ON GLUCOSE UPTAKE AND GLUCONEOGENESIS

## 4.1 INTRODUCTION

*Curcuma longa* Linn, commonly known as Turmeric, is used widely throughout the world as a flavouring and colouring in cooking, as well as for its medicinal and nutritional effects, particularly throughout the Asian countries. In the Ayurvedic Medical system, Turmeric has been used as an anti-inflammatory, antimicrobial and for many other curative properties (Ammon and Wahl: 1991). The main colouring agent of Turmeric is curcumin (giving the characteristically yellow colour to foods), and it has been shown to have a wide range of effects including antihepatotoxic (Shishodia, *et. al.*, 2005), anti-inflammatory (Srimal, 1997), hypocholesterolemic (SubbaRao *et. al.*, 1970; Patil & Srinivasan, 1971), choleretic and hydrocholagocic (Ramprasad & Sirsi, 1956), hypoglycaemic (Arun & Nalini, 2002), antioxidant (Quiles *et. al.*, 1998 Asai *et. al.*, 1999; Bengmark, 2006), anti-cancer (Huang *et. al.*, 1994; Kuo *et. al.*, 1996), antimicrobial (Gupta and Ravishankar, 2005), inhibits platelet-derived growth factor and has wound healing abilities (Kundu, *et. al.*, 2005).

Turmeric and one of its active ingredients urcumin supplemented in the diet of diabetic rats have been shown to attenuate hyperglycemia, possibly by reducing glucose influx through the polyol pathway (Arun & Nalini, 2002). Wickenberg and collaeagues (2010) in a study on healthy subjects showed that Curcumin increased postprandial serum insulin levels, but not affect plasma glucose levels, suggesting that it may have an effect on insulin secretion.

It has also been shown to delay cataracts in streptozotocin induced diabetic cataracts (Suryanarayna 2005) and decrease the renal lesions associated with

diabetes (SureshBabu & Srinivasan, 1998). An ethanol extract of Turmeric has been shown to be effective in lowering blood glucose levels in genetically diabetic KK-Ay mice, possibly through the activation of PPAR-γ receptors in adipose tissue (Kurado *et. al.*, 2005). Contrasting the above findings Curcumin has been reported to have an inhibitory effect on insulin induced GLUT4 translocation and glucose transport (Ikonomov *et. al.*, 2002), as well as cyclic AMP protein kinase (Hasmeda & Polya 1996).

There have been several studies with the use of ethanolic extracts of Turmeric and its active ingredient, curcumin (Nishiyama *et. al.*, 2005) in the management of Type 2 Diabetes in mice (Arun & Nalini, 2002; Kuroda *et. al.*, 2005), however, there has been no studies, as determined by a literature search of the Pubmed Search Engine, on the effect of aqueous extracts. Therefore, the aim of this project was to conduct a study to determine if an aqueous extract of Turmeric would increase the uptake of glucose in Swiss mice heart tissue culture; and decrease gluconeogenesis in Liver tissue culture; and look at some possible mechanisms of action.

## 4.2 MATERIALS and METHODS

Plant extraction, animal and human ethics approval, tissue preparation, and tissue culture assay and glucose analysis, clinical trial and statistical analysis have been described in Chapter 2 of this thesis.

Experiment 1:

Turmeric when added at a concentration of 10 μg/ml, in the presence of different concentrations of glucose (4, 6, 8 and 10 mM), in both heart and liver tissue culture and incubated for 24 hours to determine what effect it would have on each.

Experiment 2:

Turmeric at various concentrations (40, 20, 5, 2.5 μg/ml) along with 69.45 nmol/L insulin added to the heart tissue culture was incubated for 24 hours.

Experiment 3:

Ten μl Turmeric, Turmeric powder that had been dry roasted for 3 mins, or Turmeric boiled for 10 mins at concentrations of 40, 20, 5, 2.5 μg/ml was added to 6 mM glucose in heart and liver tissue culture and incubated for 24 hours.

Experiment 4:

A diethyl ether extraction was carried out to determine the effects on glucose uptake of the aqueous phase and the solvent phase. Ten μg/ml of the Turmeric, the solvent and aqueous phase of the extract along with a glucose concentration of 6 mM to the heart and liver tissue culture and incubated for 24 hours before glucose levels were tested.

Experiment 5:

Wortmannin was added to the heart and liver tissue cultures to determine if attenuation of uptake of glucose would occur, indicating that the insulin pathway is involved in the action of Turmeric at the PI3-K level.

Experiment 6:

Metformin at concentrations of 100 mM was added to 10 μg/ml Turmeric with glucose at a concentration of 6 mM to determine what effects the combination would have on glucose uptake in the heart and liver tissue culture.

## 4.3 RESULTS

### 4.3.1 Effect on Glucose Uptake in Heart tissue Culture and decrease in Glucose Output in Liver tissue culture

Turmeric was added to the heart tissue culture media at a concentration of 10 μg/ml in the presence of different concentrations of glucose (4, 6, 8 and 10 mM) to determine what effect it would have on each. The greatest effect was when the glucose was at 8 mM with a ($P>0.05$) significantly increased 24.02 +/- 1.25 percentage difference in uptake over the basal level. At 10 mM glucose concentration the ($P>0.05$) significantly increased the percentage uptake by 20.2 +/- 1.98%. While at 6 mM glucose there was a ($P>0.05$) significant percentage difference of 15.9 +/- 1.37%, and at 4 mM of glucose there was a negligible increase ($P>0.05$) with only a 1.97 +/- 1.25 percentage difference from basal levels (see Figure 4.1).

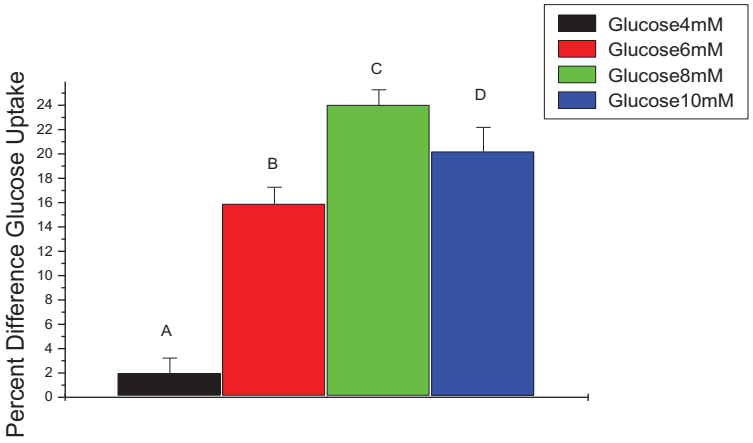



Figure 4.1: The effect of Turmeric on the glucose uptake of heart tissue in the presence of different concentrations of glucose. Values expressed as means +/- S.E. Means without common letter are significantly different.

Turmeric was added to the liver tissue culture media at a concentration of 10 µg/ml in the presence of different concentrations of glucose (4, 6, 8 and 10 mM) to determine what effect it would have on each. The greatest effect was when the glucose was at 8 mM with a (P>0.05) significantly decreased 23.2 +/- 3.6 % percentage output over the basal level. At 10 mM glucose concentration the (P>0.05) significantly decreased the percentage output by 19.7 +/- 2.9%. At 6 mM glucose there was a (P>0.05) significant percentage difference of 16.4 +/- 3.4, while at 4 mM of glucose there was a negligible decrease (P>0.05) with only a 2.8 +/- 1.2 percentage difference from basal levels (see Figure 4.2).

4. Effect of Turmeric on Glucose Uptake and Gluconeogenesis

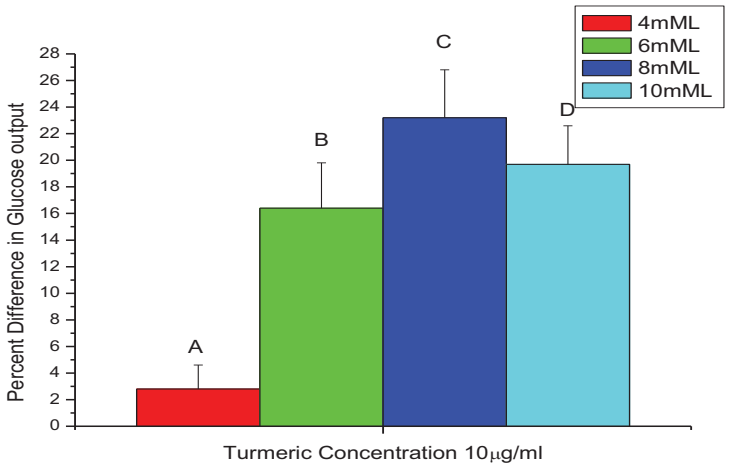



Figure 4.2: The effect of Turmeric on the glucose output of liver tissue in the presence of different concentrations of glucose. Values expressed as means +/- S.E. Means without common letter are significantly different.

Turmeric, when added to the heart tissue culture media at different concentrations (0, 2.5, 5, 10, 40 μg/ml), which also contained 69.45 nmol/L insulin showed an increase over insulin alone. Insulin alone produced an (P>0.05) significant uptake of 12.79 +/- 0.97% percent over basal. The peak increase in uptake over basal was at a concentration of 10 μg/ml of Turmeric with a (P>0.05) significant percent uptake of 38.87 +/- 1.24 %. There was a decrease in uptake though still (P>0.05) significant at 20 μg/ml and a further slight decrease at 40 μg/ml, being 36.45 +/- 1.13% and 35.24 +/- 1.21% over basal respectively. There was also a (P>0.05) significant increased uptake of 24.46 +/- 0.98% at 5 μg/ml and 17.91 +/- 1.21% at 2.5 μg/ml over basal levels. When Turmeric was added to the insulin, it was noted that there was an increase in glucose uptake over that of Turmeric alone, with the maximum increase at a concentration of 10 μg/ml of Turmeric with a (P>0.05) significant percent

uptake of 51.94 +/- 1.12%. There was a slight decrease, though still (P>0.05) significant, uptake at 20 µg/ml and a further slight decrease, though still (P>0.05) significant at 40 µg/ml, being 44.73 +/- 1.28% and 43.86 +/- 1.31% respectively. There was also an (P>0.05) significant increased uptake of 42.13 +/- 1.1% at 5 µg/ml and 32.31 +/- 1.11% at 2.5 µg/ml over basal levels and with insulin alone, it was 17.78 +/- 0.85% (See Figure 4.3).

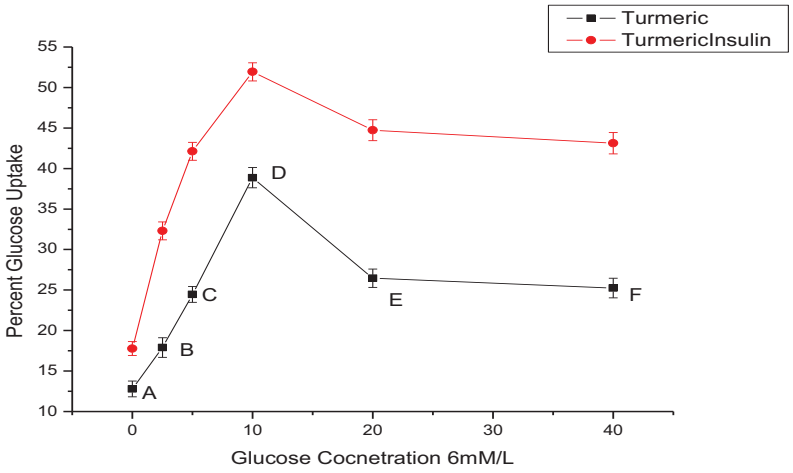



Figure 4.3: Different Concentrations of Turmeric with and without 69.45 nmol/L insulin in mouse heart tissue. Values expressed as means +/- S.E. Means without common letter are significantly different.

Turmeric when added to the heart tissue cultural media in the presence of 6 mM glucose showed an increase uptake at all concentrations, however, it was not until it reached 5 µg/ml that this was (P>0.05) significant. The peak increase in uptake over basal was at a concentration of 10 µg/ml of Turmeric with a (P>0.05) significant percent uptake of 39.4 +/-0.4% which is 18.7 +/- 0.4% over basal uptake. There was a decreased, though still (P>0.05)

significant, uptake at 20 μg/ml, and a further slight, but still (P>0.05) significant decrease at 40 μg/ml, being 16.26 +/- 0.7 and 15.34 +/- 0.8 percent, over the basal level respectively. There was also an (P>0.05) significant increase of 13.3 % +/- 2 at 5 μg/ml and 5.5% +/- 1.45 at 2.5 μg/ml over basal levels (See Figure 4.4).

When cooking Turmeric it is often dry roasted in the process to bring out the flavour and colouring and therefore the effect of dry roasting of the powder was also investigated. The dry roasted Turmeric resulted in a (P>0.05) non-significant increase in glucose uptake over the basal level at all concentrations. The greatest increased uptake being at 10 μg/ml of Turmeric, where there was an (P>0.05) non-significant increase of 4.7 +/- 0.32 percent over basal. At 5 μg/ml the percent uptake over basal was (P>0.05) non-significant, being 2.3 +/- 0.76%, while at 2.5 μg/ml there was only 0.47 +/- 0.34%, at 20 μg/ml 2.93 +/- 0.2% and at 40 μg/ml it was 2.16 +/- 0.4% (See Figure 4.4).

The Turmeric was boiled for 10 minutes, to determine if boiling for extended periods of time would influence the effect on the uptake of glucose, into the heart in tissue culture. The results indicated an increase similar to that of dry roasting, with a (P>0.05) non-significant increase in uptake with only 1.9 +/- 1.18% at its maximum, when the Turmeric was added at a concentration of 10μg/ml (see Figure 4.4).

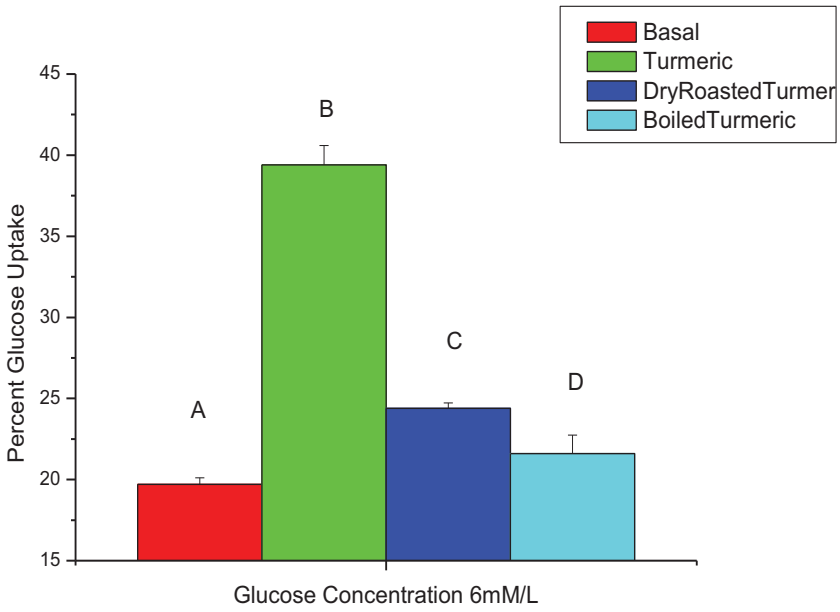



Figure 4.4: The effect of Turmeric in aqueous form, after the Turmeric powder had been dry roasted (3 Mins) and aqueous extract of the Boiled Turmeric (10 mins) on the uptake of glucose in the mouse heart muscle. Values expressed as means +/- S.E. Means without common letter are significantly different.

In the liver tissue culture plates there was an increase in the amount of glucose in the media in all wells with the liver tissue by itself increasing the amount of glucose by 9.3 +/- 1.2 % indicating that gluconeogenesis was taking place. The Turmeric showed a decreased glucose output compared to the basal at all concentrations, however, it was not until it reached 5 µg/ml that this was ($P>0.05$) significant at a concentration of 6 mM of glucose. The peak decrease in gluconeogenesis over basal was at a concentration of 10µg/ml of Turmeric with a ($P>0.05$) significant percent decrease in output of 4.21 +/- 0.03 %. There was a ($P>0.05$) significant decrease in gluconeogenesis at a concentration of 20 µg/ml Turmeric being a 6.4 +/- 1.3% output. There was also a ($P>0.05$)

significant decrease with 5 μg/ml of Turmeric which exhibited a 6.2 +/- 0.9 % output and 8.3 +/- 1 % output at a concentration of 2.5 μg/ml of the Turmeric (See Figure 4.5).

The dry roasted Turmeric resulted in a (P>0.05) non-significant decrease of glucose in the culture over the basal level at all concentrations with the greatest decrease in output being at 10μg/ml where there was a decrease of 8.91 +/- 0.04%. At 5 μg/ml the percent output over basal was a (P>0.05) non-significant 7.1+/- 0.76%. At 2.5 μg/ml the difference was only a (P>0.05) non-significant 8.83 +/- 0.34 % over basal. While that of 20 μg/ml was (P>0.05) non-significant 8.53 +/- 0.2 % over basal and at 40 μg/ml it was a (P>0.05) non-significant 2.16 +/- 0.4 % (See Figure 4.5).

The Turmeric that was boiled for 10 minutes resulted in a (P>0.05) non-significant decrease in glucose present in the culture similar to that of dry roasting with a decrease in output of only 8.71 +/- 0.03% at its maximum (see Figure 4.5).

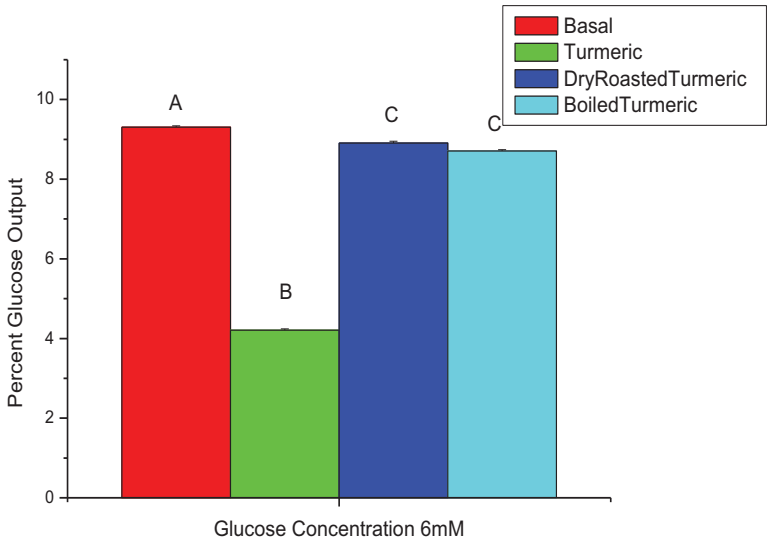



Figure 4.5: The effect of Turmeric, dried roasted Turmeric (3 mins) and Boiled Turermic (10 mins) compared to basal on the uptake of glucose on the mouse liver tissue. Values expressed as means +/- S.E. Means without common letter are significantly different.

A diethyl ether extraction was carried out to determine the effects on glucose uptake of the aqueous phase and the solvent phase by mice heart tissue. At a glucose concentration of 6 mM the heart tissue itself showed a glucose uptake of 18.6 +/- 1.4%. When 10 µg/ml of Turmeric was added the uptake showed a (P>0.05) significant increase to 38.8 +/1.8 %, while 10 µg/ml of the aqueous phase of the extraction (P>0.05) significantly increased the uptake of glucose to 62.4 +/- 1.8%. The solvent phase from this extraction (P>0.05) significantly increased the uptake to only 19.8 +/- 1.1% (see Figure 4.6).

4. Effect of Turmeric on Glucose Uptake and Gluconeogenesis

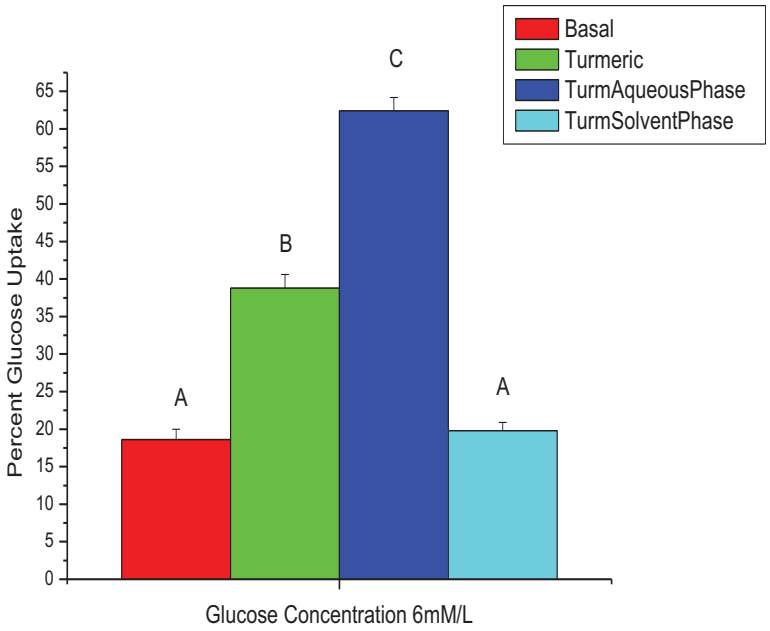



Figure 4.6: The effect of Turmeric along with the Diethyl ether extracts of Turmeric on heart tissue at a Glucose concentration of 6 mM. Values expressed as means +/- S.E. Means without common letter are significantly different.

At a glucose concentration of 6 mM the liver showed an output of glucose 9.5 +/- 1.3%. When 10 µg/ml of Turmeric was added the output decreased ($P>0.05$) significantly to 5.7 +/- 1.2 %. Ten µg/ml of the aqueous phase of a Diethyl Ether extract of Turmeric ($P>0.05$) significantly decreased the output of glucose to 3.2 +/- 1.4 %, while the solvent phase from this extraction ($P>0.05$) non-significantly decreased the uptake to only 8.6 +/- 1.2% (Figure 4.7).

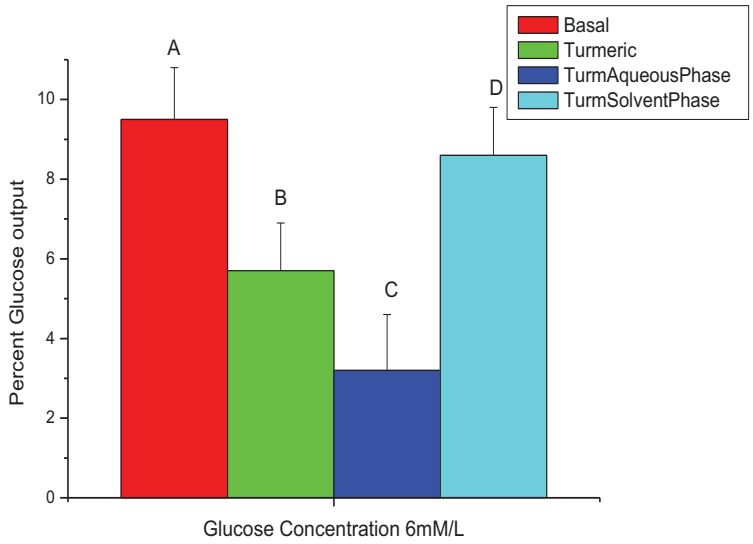



Figure 4.7: The effect of Turmeric along with the Diethyl ether extracts of Turmeric on liver tissue. Values expressed as means +/- S.E. Means without common letter are significantly different.

Wortmannin was added to the tissue cultures to determine if attenuation of uptake of glucose would occur, indicating that the insulin pathway is involved in the action of Turmeric at the PI3-K level. The basal level of glucose uptake in the heart tissue culture was 13.92 +/- 1.3%, while of insulin alone resulted in a (P>0.05) significant increased uptake of 21.56 +/- 1.2%, and when the insulin concentration was doubled, the uptake decreased, though still (P>0.05) significant to 17.3 +/- 1.4%. Turmeric alone resulted in a (P>0.05) significant increased uptake of 29.96 +/- 1.3%, while when it was added to insulin there was an (P>0.05) significant increased uptake of 52.74 +/- 1.4%. When Wortmannin was added with insulin, the level of uptake dropped back to a (P>0.05) non-significant 14.04 +/- 1.1%. When it was added to Turmeric, there was also a (P>0.05) non-significant decreased uptake from Turmeric alone to

14.32 +/- 1%, while Wortmannin on its own resulted in an uptake of 14.08 +/- 1.2% (See Figure 4.8).

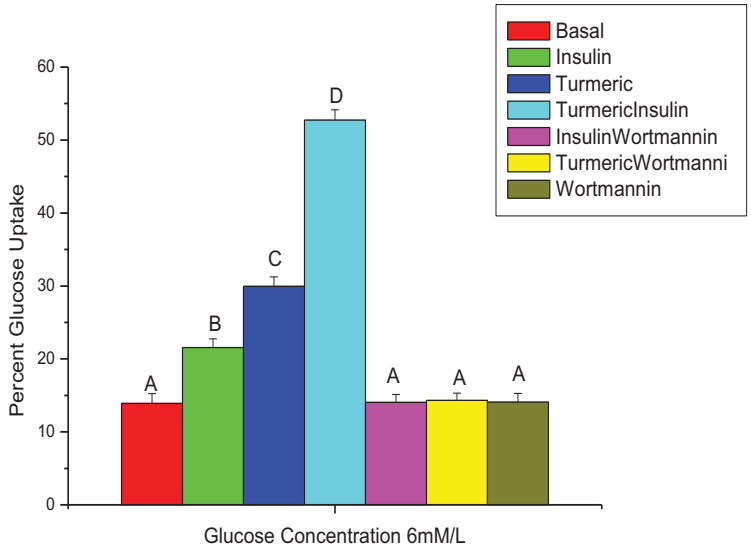



Figure 4.8: Glucose uptake with the addition of Insulin and Turmeric and the effect of the addition of Wortmannin in heart tissue culture. Values expressed as means +/- S.E. Means without common letter are significantly different.

Metformin when added to the culture media at concentrations of 100 mM along with 10 µg/ml Turmeric, at a glucose concentration of 6 mM to determine what effects the combination would have on glucose uptake in the mouse heart tissue. The basal level of glucose uptake by the heart tissue was 9.19 +/- 0.8% while that of Turmeric alone was a (P>0.05) significant 19.22 +/- 0.6% and that of 100 mM Metformin alone was a (P>0.05) significant 19.01 +/- 0.8%. When 100mM Metformin was combined with 10 µg/ml of Turmeric in the media, it showed a (P>0.05) significantly increased uptake of 17.88 +/- 0.9% (See Figure 4.9).

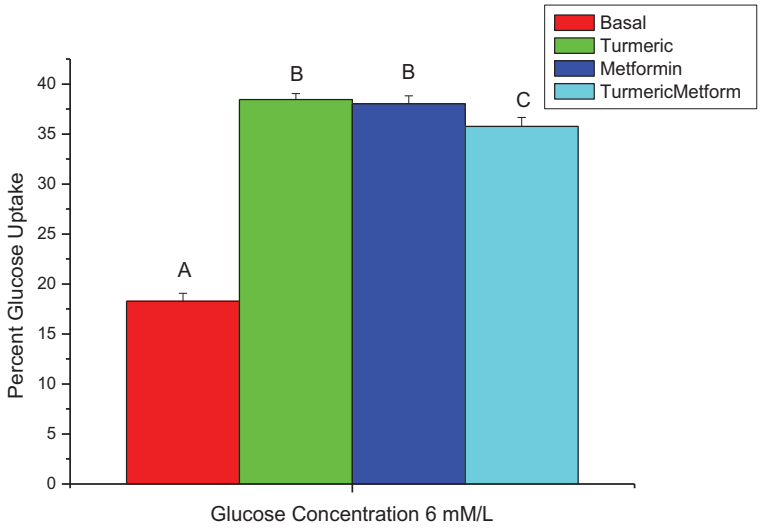



Figure 4.9: Effect of Turmeric and Metformin Glucose on heart tissue culture. Values expressed as means +/- S.E. Means without common letter are significantly different.

In the liver tissue culture with the addition of the Metformin at a concentration of 100 mM the basal level of glucose output by the liver was 9.2 +/- 1.2%, while that of Turmeric alone was a (P>0.05) significant 4.2 +/- 1.4% and that of Metformin alone was a (P>0.05) significant 4.52 +/- 1.3%. When 100 mM Metformin was combined with 10 µg/ml of Turmeric, it showed a higher but still (P>0.05) significant output of 6.94 +/- 1.3% (See Figure 4.10). Other concentrations of Turmeric and Metformin were combined to see if the toxicity effect was decreased, or this antagonistic reaction reduced, however, there did not appear to be any difference.

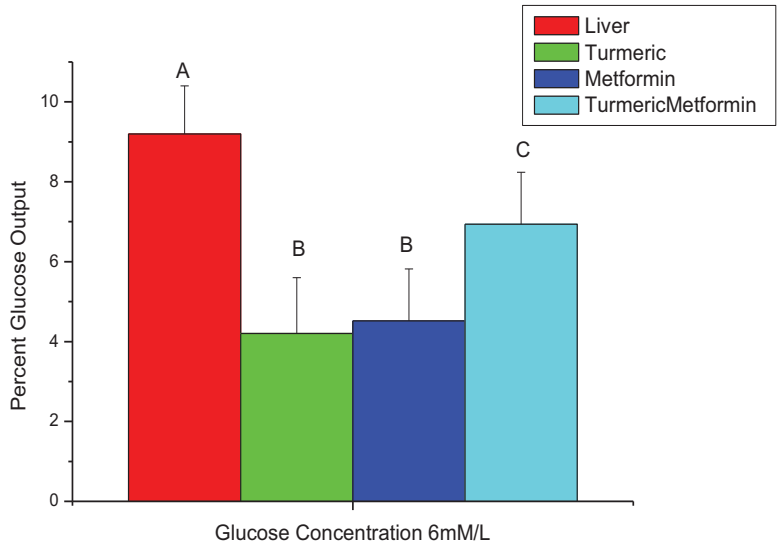



Figure 4.10: The effect of Turmeric and Metformin on liver tissue culture. Values expressed as means +/- S.E. Means without common letter are significantly different.

## 4.4 DISCUSSION

Despite numerous studies on Turmeric and one of its active constituents, curcumin, no study has been undertaken to demonstrate the effect of an aqueous extract of Turmeric on heart and liver tissue, both of which are important in glucose homeostasis. This study was undertaken to look at the effectiveness of an aqueous extract of Turmeric on Swiss mice heart and liver tissue.

Insulin resistance is a major indicator of Type 2 Diabetes, and may be present long before the FBGl show any hyperglycaemic effect, as the increased insulin output will often counteract this problem. Insulin resistance is due to the impairment of the insulin signalling cascades, glucose transporters and translocation of GLUT 4 in particular (Kellerer *et. al.,* 1999). Gluconeogenesis

also plays a major role in Type 2 Diabetes, as the liver produces glucose for cells that indicate that they are starving, and the insulin/glucagon action is not working on turning it on and off. The current management of diabetes looks at this insulin action, and mimicking its action in order to decrease BGls. This is done both by increasing the uptake of glucose by tissues throughout the body, and also decreasing gluconeogenesis. Thus, this study examined the effect of Turmeric on mice heart tissue in increasing glucose uptake, and also on liver tissue to decrease gluconeogenesis.

Mice heart tissue was used due to the ease of removal and preparation for tissue culture. A study was undertaken to determine if it exhibited the same response as Soleus muscle from the mouse and there was a similar result at all concentrations of glucose and Turmeric (data not shown). It was therefore decided to continue the use of the mice heart tissue in all of the studies.

A decision was made to look at the management of those with blood glucose levels between 5-10 nmol/L; therefore, for the tissue cultures we used 6 mM concentrations of glucose.

This study showed that there was a ($P>0.05$) significant increased uptake of glucose into heart tissue and a decrease in glucose output from the liver tissue when 5 μg/ml and 10 μg/ml of Turmeric was added to the culture. This increase was slightly higher than that of 100 mM Metformin. However, it was noted that with increased concentrations of the Turmeric there was a slight decrease, though still ($P>0.05$) significant, in uptake and increase in output, which may

have been due to experimental error, or the toxicity of the extract at higher concentrations as a crude extract was being used. Further studies would need to be carried out to determine if Turmeric at higher concentrations is toxic to the heart and liver tissue.

The decrease in the glucose uptake by the heart muscle and increased output by the liver tissue when using the dry roasted Turmeric (this is typically how it is prepared when using for curries and most cooking), and also the boiled aqueous extract was ($P>0.05$) not significant. This indicates that very high heat will deactivate the active ingredient/s suggesting the active ingredient/s are proteins. It also suggests that the eating of curries etc, a popular winter food throughout the many counties where Type 2 Diabetes is on the increase, will not aid in the increase of glucose uptake even without looking at the absorption factors.

A diethyl ether extraction was carried out to look at what type of chemical might be involved in the increased uptake of glucose by the heart muscle tissue and glucose output by the liver. The study showed that there was a ($P>0.05$) significiantly greater uptake by the heart muscle and decreased glucose output by the liver when the aqueous phase was used rather than with the whole aqueous extract. There was little, if any, increase in uptake or decrease in output over basal, when the solvent phase was used. Curcumin, the chemical that has been shown to be effective in lowering glucose levels in previous studies (Hasmeda & Polya 1996; Arun & Nalini, 2002; Ikonomov *et. al.,* 2002; Korado

*et. al.,* 2005; Nishiyama *et. al.,* 2005) would be found in the solvent phase, indicating that there is a different compound that is bringing about the increased glucose uptake. Considering the loss of activity when the Turmeric solution was boiled or the Turmeric was dry roasted before the aqueous extract was made, it also suggests that the active component is a protein. Interestingly the aqueous phase increased the uptake to a greater extent than that of the straight Turmeric, suggesting that there is an ingredient in the extract that may be inhibiting the uptake, or that the more purified sample was more efficient. Previous studies have shown that Curcumin has an effect on decreasing BGLs, however, this study shows that the aqueous phase is producing a different active constituent which is having a similar effect. Further studies would need to be carried out to isolate this compound that is bringing about the increase in glucose uptake by the mouse heart tissue.

The differences in glucose uptake by the mouse heart tissue and deceased output by the liver over basal at different concentrations of glucose indicates that Turmeric has little or no effect when the glucose level is within normal range. However, it increases the uptake and decreases the output when glucose levels are high, indicating that the taking of Turmeric will not lead to hypoglycaemia, coma and possible death. Very high levels of glucose were not tested as it was out of the scope of this study and therefore the effect on higher glucose levels needs further studies to determine the Turmeric's effectiveness at these concentrations.

To undertake a preliminary study looking at the mechanism of action a maximum dose response of insulin (69.45 nmol/L) and also Wortmannin, which blocks the insulin pathway at the PI3-K level by inhibiting PI3-kinase (Hausdorf *et. al.,* 1999) was added to the Turmeric treated heart tissues. The enhanced uptake when Turmeric and insulin are combined and the decrease when Turmeric is added at higher concentrations indicate that the Turmeric may be acting via an insulin-mediated peripheral glucose pathway. This is similar to the pathway used by the hypoglycaemic drug, Metformin (Bailey, 1988). The inhibitory effect of Wortmannin on Turmeric supports the theory that the Turmeric is acting via the classical PI3-kinase pathway.

Today many patients who have Type 2 Diabetes are prescribed Metformin in the early stages of the disease progression and therefore the interaction between Turmeric and Metformin was investigated. When Turmeric and Metformin were placed in heart or liver tissue culture together there was an antagonistic effect which may be due to increased toxicity of the Turmeric/Metformin or experimental error with the use of a crude extract of Turmeric. This may have been due to a drug interaction that needs to be explored further.

## 4.5 CONCLUSIONS

An aqueous extract of Turmeric increases the uptake of glucose in mice heart tissue culture and decrease gluconeogenesis from the mouse liver in a dose

dependent manner, with the optimum concentration being 10 μg/ml. It also tended to be slightly toxic at higher levels with a decrease in glucose uptake by heart muscle tissue culture and increased gluconeogenesis in liver tissue culture over that of the optimum concentration; however, there still was an increase over the basal rate. The toxicity may be due to the crude extract that was used or that some active ingredient comes into effect at the higher concentrations so there can be seen both this toxic effect to the heart and liver tissue as well as an increased up take of glucose. Dry roasting or boiling of the Turmeric decreases its ability to increase the uptake of glucose in the mouse heart tissue or decrease the glucose output in liver tissue culture. This was most likely due to deactivation of active constituents suggesting that the active constituent/s is a protein. This is supported by the diethyl ether solvent extract where it is noted that the aqueous phase increased the uptake of glucose in heart tissue and decreased output in the liver tissue while the solvent phase did not. The addition of insulin and Wortmannin indicates that the Turmeric was acting via the insulin pathway as it is blocked by the Wortmannin at the PI3K level. However, it is interesting to note that there is an additive effect to insulin at all concentrations of Turmeric and so suggests entering the insulin pathway at a different point then glucose-6-phosphate. There is an antagonistic reaction when Turmeric and Metformin are added together to the heart and liver tissue cultures, therefore, possibly should not be used in conjunction with each other; however, much more research needs to be carried out to determine this interaction.

In summary, Turmeric does appear to increase glucose uptake in the mouse heart muscle and decrease gluconeogenesis in the liver. The active ingredient appears to be a protein however isolation studies need to be undertaken to determine exactly what it is. The active ingredient appears to be working through the insulin pathway and becomes toxic on the addition of Metformin.

There needs to be further studies carried out to determine Turmeric's active ingredient/s and their mechanism/s of action.

5. Effect of Glucostat on Glucose Uptake and Gluconeogenesis

# **5.** EFFECT OF GLUCOSTAT ON GLUCOSE UPTAKE AND GLUCONEOGENESIS

## 5.1 INTRODUCTION

Glucostat is a combination of the Chinese herbs including *Trichosanthes kirilowii* (1900 mg), *Polygonatum sibiricum* (714.5 mg), *Dioscorea oppostia* (286 mg), *Panax ginseng* (95 mg), and *Stevia rebaudiana* (47.6 mg) per capsule that was available from Metagenics, Healthworld Ltd., Eaglefarm, Queensland, Australia, 4009, for the management of Type 2 Diabetes. After a search of the literature it was found that there has been some research undertaken on 4 of the individual herbs, but only one (Collins & McFarlane, 2006) on the combination, sold as Glucostat.

Panax ginseng from the *Panax quinquefolius* plant is used as an adaptogen, aphrodisiac and nourishing stimulant (Ren, 1986). It has been reported to have many uses, including antidepressant, a demulcent, a diuretic, a sedative and aids in concentration and memory (Fetrow & Avila 2001). A study by Sotaniemi and colleagues (1995) and Vuksan and collegues (2001) showed that it decreased fasting BGL in both diabetic and non-diabetic patients, and enhanced insulin release from the pancreas. It alone increased the number of insulin receptors as well as aided in hepatic dysfunction, and hyperlipidemia. Suzuki (1989) showed a reduction in insulin resistance and an increase in the number of insulin receptors in mice, while Wallace (1999) showed a reduced fasting BGL after the administration of Panax ginseng to humans. The effect of Panax ginseng seems to be dependent on the time taken, with the most effective time being 40 minutes

prior to ingestion of a meal (Vuksan *et. al.,* 2001). In a human trial, Vuksan and colleagues (2000b) showed that no more than 3 gm of American ginseng was required to decrease fasting blood glucose levels, irrespective of the time taken before the meal.

Sotaniemi and colleagues (1995) suggested that an unspecified species of ginseng improved mood and psychophysical performances, which led to beneficial lifestyle changes, such as diet and increased physical activity; this may be useful in the management of Type 2 Diabetes. They also suggested that there was a direct action as well, in that there was improved insulin sensitivity, and increased glycogen storage, independent of diet and physical activity. Suzuki and colleagues (1991) showed that an unspecified ginseng inhibited gastric secretion in rats, while Onomura and colleagues (1999) have shown that it decreases glucose and maltose absorption in isolated human and rat duodenum. Ohnishi and colleagues (1996) found that Chinese ginseng increased GLUT2 in the livers of normal and diabetic mice, and stimulated insulin secretion in mice islets and rat pancreas.

The active components of ginseng are believed to be the ginsenosides, a group of steroidal saponins (Huang 1999; Attele *et. al.,* 2002), which are distributed in the roots, leaf and berry of the plant. Different parts of the plant have a distinct ginenoside profile (Attele *et. al.,* 1999) and may have different pharmacological activity. Attele and colleagues (1999) showed that Panax ginseng berry significantly improved systemic insulin sensitivity and glucose

homeostasis in mice. However, Dey and colleagues (2003) showed that Panex ginseng berry exhibited more potent anti-diabetic activity than did the root, and only the berry exhibited anti-obesity effects in ob/ob mice. The gensenoside Re plays a role in this action; however, was not associated with body weight changes, suggesting that there may be other active constituents as well. Adenosine, which enhances lipogenesis (Okuda & Yoshida, 1980), as well as a carboxylic acid which also enhanced lipogenesis (Sekiya & Okuda, 1981), have been identified from an aqueous extract of *Panax ginseng*; however, hypoglycaemic activity was not tested. Kimura and colleagues (1981) isolated DPG-3-2, a water extract and EPG-3-2, a methanolic extract from *Panax ginseng,* with EPG-3-2 and DPG-3-2 increasing serum insulin levels in diabetic mice, yet had no effect on normal mice; however, DPG-3-2 had an additive effect on insulin release in both diabetic and normal rats. There has also been 5 glycans (Panaxan A-E) isolated from the roots of ginseng (Konno *et. al.,* 1984) which exhibited hypoglycaemic activity in both normal and alloxan-induced diabetic mice.

There have been a few side effects reported from the administration of American ginseng including nausea, headache, dizziness, insomnia, nervousness and hypertension (Miller, 1998), along with diarrhoea and fatigue which have been reported in a few isolated cases (reviewed in Vogler *et. al.,* 1999). However, with each of these reported side effects it is not sure if the American

ginseng was the cause or if other factors such as diet, health status at the time etc was involved.

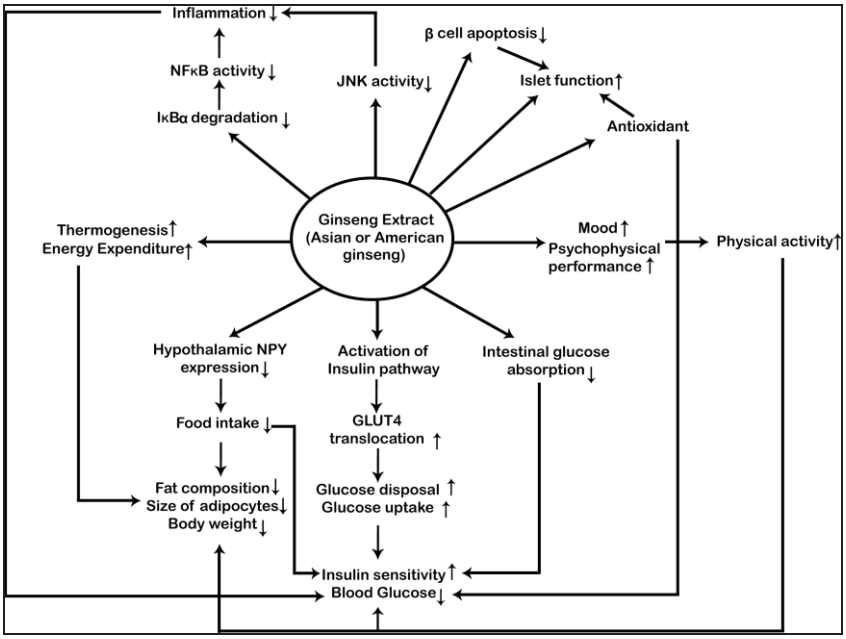



Figure 5.1: Ginseng is able to improve glucose metabolism through multiple pathways: 1) Glucose disposal is increased partially due to activation of insulin signalling pathway and GLUT 4 translocation by ginseng; 2) Ginseng is able to suppress food intake through inhibition of NPY expression in the hypothalamus; 3) Physical activity is increased through improvement of mood and psychophysical performance; 4) Fat composition and body weight are reduced partially related to up-regulation of thermogenesis and energy expenditure; 5) Antioxidant and anti-inflammatory effect may be involved in the mechanisms of insulin sensitization; 6) protects islet function through antioxidant and inhibition of β-cell apoptosis. (Yin *et. al.,* 2008).

*Stevia rebaudiana* (Bertoni) is a perennial shrub of the Asteraceae (Compsoitae) family, native to certain regions of South America. It is approximately 300 times sweeter than sucrose and has been used for several years as a sweetener in South America, Asia, Japan, and China and in some countries of Europe (Geuns, 2003), although it does leave a bitter after taste. In Brazil, Korea and Japan, Stevia leaves, steviosoide and highly refined extracts

are officially used as a low-calorie sweetener (Kim *et. al.,* 2002; Mizutani and Tanaka, 2002). The sweet components are believed to be stevioside, steviolbioside and various rebaudiosides and dulcoside, which occur mainly in the leaves (Geuns, 2003).

The *Stevia rebaudiana* plant and its constituents have had a controversial history as far as its antidiabetic effects are concerned (White 1994). Curie (1986) showed a decreased BGL after administration of 20 gm/day to a Type 2 Diabetic GK rat, and postulated it was due to an increase in the mitochondrial respiration rate; or inhibition of gluconeogenesis. As well as an antihyperglycaemic, insulinotropic and glucagonostatic effect, it is thought that this might be due to a direct action on β-cells (Jeppesen et al. 2000). Experiments performed by Malaisse and colleagues (1998) in rats, showed that in the presence of stevioside there was an increase of insulin output. However, it only reached a statistically significant effect at the higher concentrations, as well as acting as a stimulant on hepatic glycogen synthesis under gluconeogenic conditions. Human trials, using Stevioside, were carried out by Curi (1986), which showed a significant decrease in BGL by increasing glucose tolerance.

There is controversy over whether *Stevia rebaudiana,* taken as a supplement, will have any effect in decreasing FBG as it has been shown that Stevioside, one of its main active ingredients taken orally, is not absorbed or only in very small amounts in the human (Bracht *et. al.,* 1985; Yamamto *et. al.,* 1985; Koyama *et. al.,* 2003), and none of the digestive enzymes of man and

most animals are able to degrade stevioside into its aglycone, steviol (Wingard *et. al.,* 1980; ; Hutapea *et. al.,* 1997; Koyama *et. al.,* 2001; Koyama *et. al.,* 2003). However, Stevioside is metabolised to steviol by digestive tract bacteria and this then is possibly able to be absorbed (Koyama *et. al.,* 2003). It has been shown that the bacteria in the human gastrointestinal tract are able to transform stevioside into steviol *in vitro*; however, it has not been proven that this occurs *in vivo,* nor that it is absorbed by the intestines directly into the blood stream (Hutapea *et. al.,* 1997; Koyama *et. al.,* 2001). Experiments by Wingard and colleagues (1980) and Nakayama and colleagues (1986) and Koyama and colleagues (2003) have shown that steviol can be absorbed by the intestines.

There has been some controversial studies concerning the effect of stevioside on fertility with Planas and Kuae (1968) reporting a decrease of live birth rates in rats; however, this was refuted by Shiotsu (1996) who carried out more reliable experiments using many more animals. Melis (1999) suggested a possible decrease in male fertility, whereas Oliveira-Filho (1989) and colleagues using the same stevioside dosage, which were very high – 5.3 gms stevioside/kg body weight - did not find this. Stevioside also does not interfere with the uptake of other nutrients such as vitamins and minerals from the intestine (Wood *et. al.,* 1996; Geuns *et. al.,* 2003).

Gua lou, from the root tubers of the *Trichosanthes kirilowii* plant, has been used in Chinese medicine for the ‘Thirsting Disease’ for centuries. Hikino and colleagues (1989) found that the non-dialyzable portion decreased BGL in mice

and they isolated five glycans (Trichosans A, B, C, D, and E), all of which showed hypoglycaemic actions in normal mice and Trichosan A decreased BGL in alloxan-induced hyperglycaemic mice. A galactose-binding lectin from the roots of the plant stimulate the incorporation of D-[3-3H]glucose into lipids in adipocytes (Ng *et. al.,* 1985); however, it did not inhibit lypolysis in the manner of insulin inhibiting hormone-induced lipolysis (Ng *et. al.,* 1985; Wong *et. al.,* 1985). *Trichosanthes kirilowii* has been used for centuries as an abortificant in Chinese medicine and Lu and colleagues (2001) believe that this is due to its ribosome inhibiting property. Xu and colleagues (2001) have shown that *Trichosanthes kirilowii* also has anti-inflammatory properties, and this may be the process that aids in the management of Type 2 Diabetes, as it has now been shown that inflammation and Type 2 Diabetes are linked (Festa *et. al.,* 2002; Freeman *et. al.,* 2002). Yeung and colleagues (1987) isolated an immunosuppressive protein from the root of the *Trichosanthes kirilowi* which inhibited ConA-induced transformation in lymphocytes isolated from spleens of mice, and this protein was a potent inducer of mid-term abortion in mice. This was supported by Gong and colleagues (2008) when they showed that trichosanthin may act as an anti-rejection therapy.

Shan yao from the *Dioscorea opposita* plant has also been used for centuries in Traditional Chinese Medicine. It is one of the herbs used in Die-Huang-Wan, often a prescribed treatment for diabetes in China and Japan as well as other Asian countries. It has been shown to increase insulin secretion in

normal rats as well as decrease FBGls; however, has no effect on streptozotocin-induced diabetic rats (Hsu *et. al.,* 2007). Hsu and colleagues (2007) showed that Die-Huang-Wan would decrease the plasma glucose levels in fructose rich chow fed rats. *Dioscorea opposite* appeared to be the herb responsible for this, as, when it was removed from the combination, no increase in insulin secretion or decrease in FBG would result. Where as if other herbs were removed from the combination then no lowering of the plasma glucose levels would result, indicating that *Dioscorea opposite* is responsible for the increase in whole body insulin sensitivity. Hao and colleagues (1991) showed that there was an anti-hyperglycemic effect in mice and suggested that the active ingredients were polysaccarides.

Huangjing or Siberian Solomon's Seal is from the *Polygonatum sibiricum* plant and has been used in Traditional Chinese Medicine for centuries for the treatment of wasting and thirsting disease. Kato and Miura (1993) have shown that a methanol extract of *Polygonatum sibiricum* decreased blood glucose without any changes in serum insulin levels, in normal and streptozotocin-induced diabetic mice. A later study by Kato and colleagues (1994) showed that an intraperitoneal injection of a methanol extract of a *Polygonatum sibiricum* decreased glucose output by the liver in Wistar Fatty rats, most likely by decreasing GLUT mRNA and associated proteins. Kato and Miura (1993) isolated an active constituent from the *Polygonatum sibiricum* which was the spirostanol glycoside, PO-2; Ahn and colleagues (2006) have isolated 4 steroidal

saponins from a methanol extract, the neosibiricosides A-D (1-4), which they tested for cytotoxicity against cultured human MCF-7 breast cancer cells, and showed that 3 and 4 had moderate cytotoxic activity while 1 and 2 had none.

In this study we looked at the mechanism of action for the effectiveness in glucose uptake in mouse heart tissue and decreasing gluconeogenesis as there was a significantly increased glucose uptake in heart mouse tissue culture in the screening process that was carried out and discussed in chapter 3.

## 5.2 MATERIALS AND METHODS

Plant extraction, animal ethics approval, tissue preparation, and tissue culture assay and glucose analysis, clinical trial and statistical analysis have been described in Chapter 2 of this thesis.

Experiment 1:

Glucostat (10 µg/ml) or Boiled Glucostat (10 min) at a concentration of 10 µg/ml and in the presence of different concentrations of glucose (4, 6, 8 and 12 mM) was added to heart or liver tissue culture and incubated for 24 hours.

Experiment 2:

Glucostat and Boiled Glucostat (10 min) at various concentrations (40, 20, 5, 2.5 µg/ml) in the presence of 6mM glucose was added to heart or liver tissue culture and incubated for 24 hours.

Experiment 3:

A diethyl ether extraction was carried out to determine the effects on glucose uptake of the aqueous phase and the solvent phase. Ten μg/ml of the Glucostat, the Boiled Glucostat (10 min) and the solvent and aqueous phase of the extract in the presence of 6 mM glucose was added to the heart or liver tissue culture and incubated for 24 hours.

Experiment 4:

Wortmannin was added to the heart and liver tissue cultures to determine if attenuation of uptake of glucose would occur indicating that the insulin pathway is involved in the action of Turmeric at the PI3-K level.

Experiment 5:

Metformin at concentrations of 100 mM was added to 10 μg/ml Glucostat and Boiled Glucostat (10 min) in the presence of 6 mM glucose to determine what effects the combination would have on glucose uptake in the heart and liver tissue culture.

### 5.2.1. Sheep Studies.

Merino wethers (6) were utilized as the animal model in this study. They were housed at the University if New England, Armidale, NSW, Australia and remained in an open paddock until required for experimentation. Throughout the experimentation period they, were housed in 12 $m^2$ pens and were allowed ad lib feeding of a blend of White chaff and Lucerne, along with water. The wethers were weighed at the beginning of the experiment and randomly divided

into 3 groups; control, oral treatment and IV treatment, to nullify the influence of age and weight.

The first group were administered the equivalent of 1 Glucostat capsule containing (*Trichosanthes kirilowii* (1900 mg), *Polygonatum sibiricum* (714.5 mg), *Dioscorea oppostia* (286 mg), *Panax ginseng* (95 mg), and *Stevia rebaudiana* (47.6 mg) dissolved in water by 50 ml drenching syringe, 3 times per day. The second group received the same dosage which had been soaked in 0.9% sodium chloride administered via jugular catheter, 3 times per day. The control group had 50ml of saline injected via the jugular catheter, 3 times per day. Each merino received treatments at 8 am, 12 noon and 6 pm for 4 days.

After the 4 days, blood samples were taken from each animal to determine the basal glucose level. An intravenous dose of glucose (240 mg) was administered to commence a Glucose Tolerance Test. Blood samples were taken at 5, 10, 15, 30, 45, 60, 90 and 120 minutes and the plasma collected.

#### 5.2.1.1 Collection of Plasma Samples

Each sheep was fitted with an intravenous catheter inserted into either the left or right jugular vein. Blood was collected in 10 ml polypropylene tubes, each containing 20 μL of Lithium heparin (1000 I.U./ml). Immediately after the sample was taken, the catheter tubing was flushed with 3.8% Sodium Citrate as an anticoagulant agent. Samples were then centrifuged at 2500-3000 rpm for 20 minutes. The plasma was removed and transferred to labelled 5 ml sample vials and stored at -20$^{o}$C until required for assaying.

### 5.2.1.2 Plasma Analysis

The frozen plasma samples were thawed at room temperature and 150-200 μL of each sample of plasma was analysed using the Dade Chemical analyser (DADE-XL, Dupont, USA) to determine the total concentration of glucose per sample in mmol/L.

## 5.3 RESULTS

### 5.3.1 Effect of Glucostat on Glucose Uptake in Mouse Heart and Glucose Output by Liver tissue culture

Glucostat was added to the heart tissue culture media at a concentration of 10 μg/ml in the presence of different concentrations of glucose (4, 6, 8 and 10 mM) to determine what effect it would have on each. The greatest effect was when the glucose was at 8 mM with a ($P>0.05$) significant percentage difference in uptake of 17.4 +/- 0.87% over the basal level. At a glucose concentration of 12 mM there was a ($P>0.05$) significant difference of 16.1 +/-1.2%, and at 6 mM glucose there was a ($P>0.05$) significant percentage difference of 10.91 +/- 0.9%. At a glucose concentration of 4 mM there was a ($P>0.05$) negligible increase with only a 2.4 +/- 1.2% difference from basal levels (See Figure 5.2).

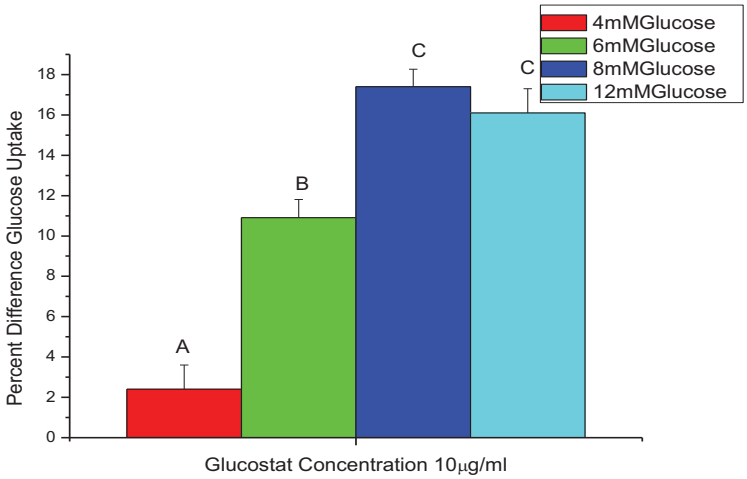



Figure 5.2: The effect of Glucostat added to different concentrations of glucose on heart tissue showing the difference in glucose uptake over basal. Values expressed as means +/- S.E. Means without common letter are significantly different.

Glucostat was added to the liver tissue culture media at a concentration of 10 μg/ml in the presence of different concentrations of glucose (4, 6, 8 and 10 mM) to determine what effect it would have on each. The greatest effect was when the glucose was at 8 mM with a (P>0.05) significantly decreased output of 11.51 +/- 1.9% over the basal level. At a glucose concentration of 12 mM the (P>0.05) significant percentage difference was 10.76 +/- 2.11%; and at 6 mM glucose there was a (P>0.05) significant percentage difference of 10.94 +/1 2.14%. At 4 mM of glucose there was a (P>0.05) negligible difference with only a 2.9 +/- 1.83% difference from basal levels (See Figure 5.3).

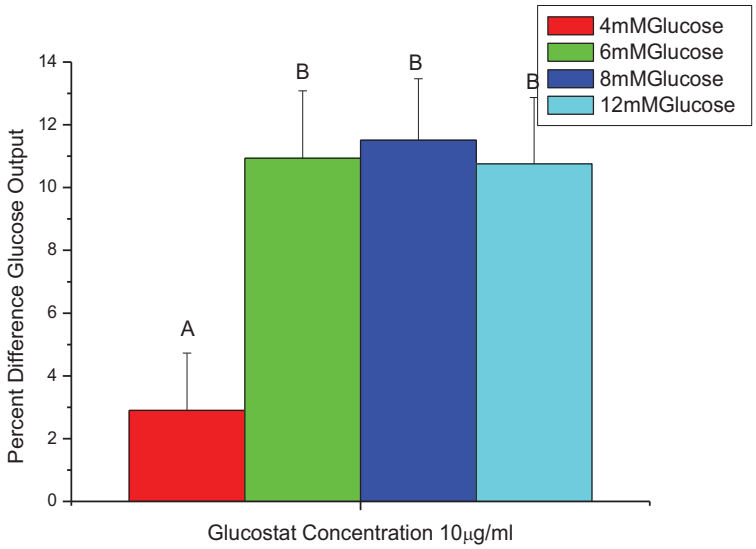



Figure 5.3: The effect of Glucostat added to different concentrations of glucose on mouse liver tissue showing the difference in glucose uptake over basal. Values expressed as means +/- S.E. Means without common letter are significantly different.

When the Glucostat was boiled and added to different concentrations of glucose in the heart tissue culture media the greatest increase of uptake was at a glucose concentration of 8 mM glucose with a (P>0.05) significant percentage difference in uptake of 15.8 +/- 0.87 over the basal level. At a glucose concentration of 12 mM there was a (P>0.05) significant percentage difference of 14.3 +/- 0.9%; and at a glucose concentration of 6 mM there was a (P>0.05) significant percentage difference of 8.91 +/- 1.1%. At glucose concentration of 4 mM there was a (P>0.05) negligible increase with only a 2.1 +/- 1.2% percentage difference from basal levels (see Figure 5.4).

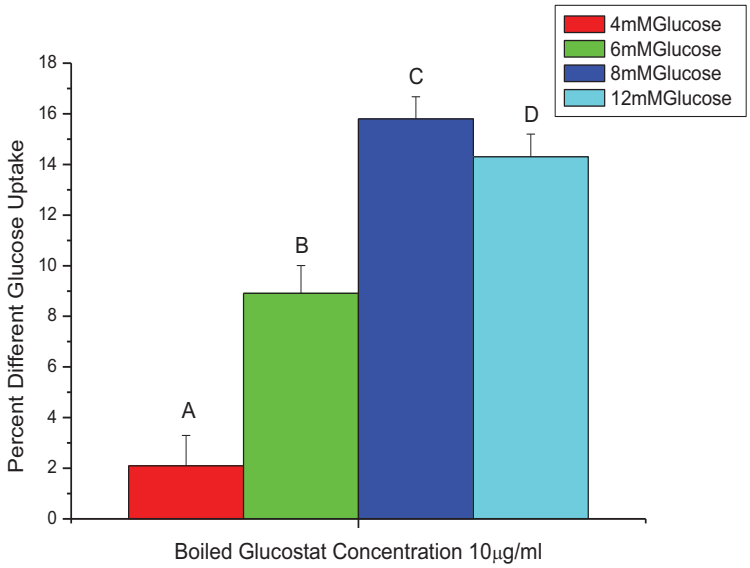



Figure 5.4: The effect of Boiled Glucostat added to different concentrations of glucose on heart tissue showing the difference in glucose uptake over basal. Values expressed as means +/- S.E. Means without common letter are significantly different.

Ten μg/ml of Boiled Glucostat was added with different concentrations of glucose (4, 6, 8 and 12 mM) to determine what affect it would have on each and if the boiling of the Glucostat would affect the results. The greatest effect was with a glucose concentration of 8 mM which showed a (P>0.05) significant percentage difference of 9.34 +/- 1.76%. At a glucose concentration of 12 mM there was a (P>0.05) significant percentage difference of 8.83 +/- 1.97%; while at a glucose concentration of 6 mM there was a (P>0.05) significant percent difference of 8.96 +/- 1.87%. At a glucose concentration of 4 mM there was a (P>0.05) negligible increase with only a 2.7 +/- 1.54% difference from basal levels (See Figure 5.5).

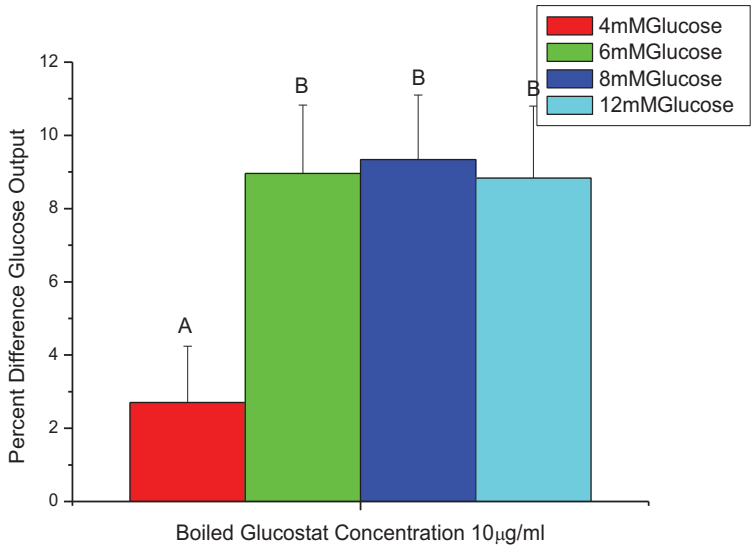



Figure 5.5: The effect of Boiled Glucostat added to different concentrations of glucose on mouse liver tissue showing the difference in glucose output over basal. Values expressed as means +/- S.E. Means without common letter are significantly different.

Glucostat when added to the heart tissue cultural media showed an increased uptake at all concentrations; however, was at a concentration of 10 µg/ml of Glucostat with a (P>0.05) significant percentage uptake of 16.87 +/- 1.13 %. There was a slight decrease, though still (P>0.05) significant percentage uptake at 20 µg/ml of 15.29 +/- 1.14%. There was also an (P>0.05) significant increased uptake at 5 µg/ml Glucostat of 16.01+/- 1.15% and at 2.5 µg/ml Glucostat there was an increase of 11.67 +/- 1.21 % over basal levels (See Figure 5.6). The Boiled Glucostat resulted in an increased uptake at all concentrations. The highest uptake was at a concentration of 20 µg/ml with an (P>0.05) significant increase of 16.54 +/-1.14%, while at a concentration of 10µg/ml there was an (P>0.05) significant increased uptake of 16.14 +/- 1.15%. At a concentration of 5 µg/ml the uptake was also (P>0.05) significant at 11.74

+/- 1.02 % and at a concentration of 2.5 µg/ml there was a (P>0.05) significant increase in glucose uptake was 7.58 +/- 1.23% (See Figure 5.6).

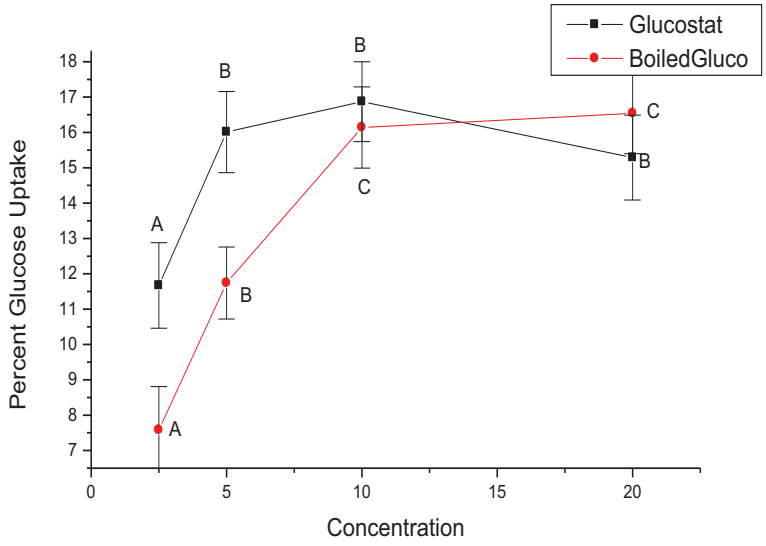



Figure 5.6: The effect of Glucostat, Boiled Glucostat (10 mins) compared to basal on the uptake of glucose in the mouse heart muscle. Values expressed as means +/- S.E. Means without common letter are significantly different.

In the liver tissue culture there was an increase in the amount of glucose by 12.73 +/- 1.2%, indicating that gluconeogenesis was taking place. The Glucostat resulted in a decreased the amount of glucose output, over the basal levels, at all concentrations, however, it was not until it reached 5 µg/ml that this was (P>0.05) significant. The peak decrease in glucose was at a Glucostat concentration of 10 µg/ml of Glucostat with a (P>0.05) significant percentage output of 5.38 +/- 1.4%. There was a decrease in glucose output at a Glucostat concentration of 20 µg/ml with a (P>0.05) significant uptake of 7.27 +/- 1.2%. There was also a decrease with a Glucostat concentration of 5 µg/ml which

exhibited a (P>0.05) significant output of 6.97 +/- 1% output and at a concentration of 2.5 µg/ml there was a (P>0.05) non-significant decreased output of 8.02 +/- 1.3% (See Figure 5.7).

The Boiled Glucostat resulted in a peak decrease in glucose output at a concentration of 20 µg/ml with a (P>0.05) significant percentage output of 3.77 +/- 0.9%. There was a decrease in glucose output at a concentration of 10 µg/ml Boiled Glucostat with a (P>0.05) significantly decreased output of 5.07 +/- 1.2 %. There was also a (P>0.05) significant decrease with 5 µg/ml of Boiled Glucostat decreased out put of 8.13 +/- 1.4% and at a concentration of 2.5 µg/ml there was a (P>0.05) negligible decrease of 11.58 +/- 1.2 % (See Figure 5.7).

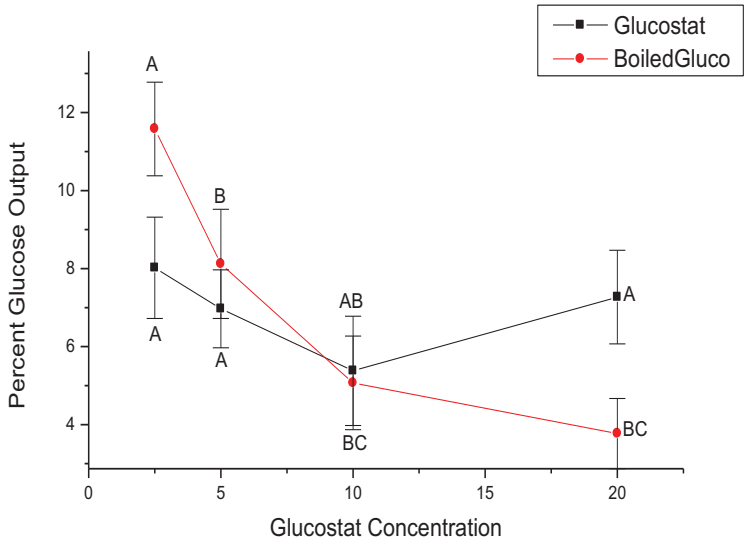



Figure 5.7: The effect of Glucostat and Boiled Glucostat (10 mins) compared to basal on the output of glucose in the mouse liver tissue. Values expressed as means +/- S.E. Means without common letter are significantly different.

5. Effect of Glucostat on Glucose Uptake and Gluconeogenesis

A solvent extraction was carried out to determine the effects on glucose uptake of the aqueous phase and solvent phase on heart and liver tissue. At a glucose concentration of 6 mM the heart tissue culture showed an uptake of glucose from the media of 18.6 +/- 1.4 %. When 5 μg/ml of Glucostat was added the uptake (P>0.05) significantly increased to 28.8 +/- 1.6 %; while with the same amount of the Boiled Glucostat the uptake was only slightly lower, though still significant, being 25.4 +/- 1.4%. Five μg/ml of the solvent phase of the Glucostat (P>0.05) significantly increased the uptake of glucose to 29.9 +/- 1.8 %, while the aqueous phase from this extraction (P>0.05) significant increased the uptake to 29.8 +/- 1.1%.

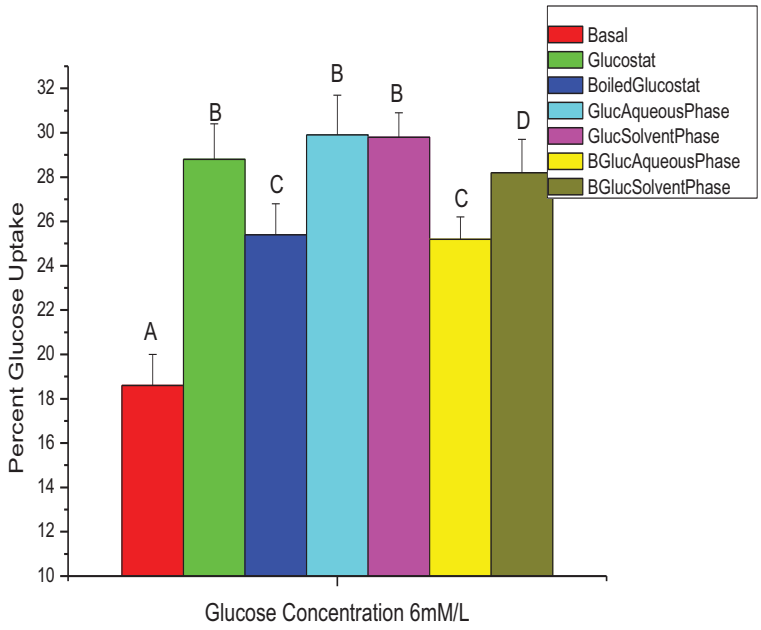



Figure 5.8: The effect of Glucostat and Boiled Glucostat along with their Diethyl ether extracts on mouse heart tissue. Values expressed as means +/- S.E. Means without common letter are significantly different.

The aqueous phase from the Boiled Glucostat solution (P>0.05) significantly increased the glucose uptake to 25.2 +/- 1.0 % and the solvent phase to 28.2 +/- 1.5 % (See Figure 5.8).

At a glucose concentration of 8 mM the liver showed an output of glucose 12.76 +/- 1.2%. When 5 µg/ml of Glucostat was added the output (P>0.05) significantly decreased to 6.87 +/- 1.1%, while with the same amount of the Boiled Glucostat the output was 8.13 +/- 1%. Five µg/ml of the solvent phase of the Glucostat (P>0.05) significantly decreased the output of glucose to 5.76 +/- 1.3%, while the aqueous phase from this extraction (P>0.05) significantly decreased the output to 7.75 +/- 1%. The solvent phase from the Boiled Glucostat solution (P>0.05) significantly decreased the glucose output to 6.01 +/- 1.1% and the aqueous phase to 6.29 +/- 1.2% (See Figure 5.9).

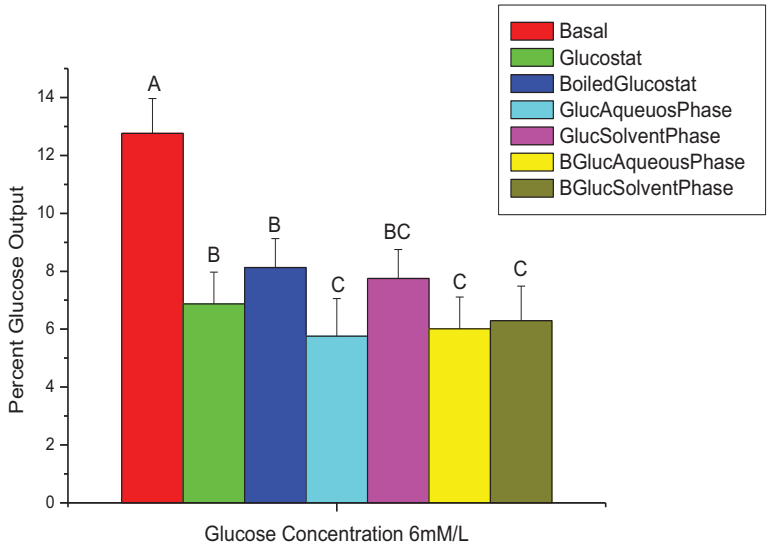



Figure 5.9: The effect of Glucostat and Boiled Glucostat along with the Diethyl ether extracts on liver tissue culture. Values expressed as means +/- S.E. Means without common letter are significantly different.

Wortmannin was added to the tissue cultures to determine if attenuation of uptake of glucose would occur, indicating that the insulin pathway is involved in the action of Glucostat at the PI3-K level. Sixty nine.forty five nmol/L of insulin alone resulted in a 10.74 +/- 0.9% uptake of glucose while the basal level was 6.98 +/- 1.2%. Ten µg/ml of Glucostat alone (P>0.05) significantly increased the glucose uptake to 10.07 +/- 1.0%. The maximum dosage of insulin was added with the 10 µg/ml Glucostat resulting in a (P>0.05) significant uptake of 11.4 +/- 1.4%. On the addition of Wortmannin to the culture with insulin the glucose uptake was 6.87 +/- 0.8%, while when the Wortmannin was added to the Glucostat culture there was a (P>0.05) significant uptake of 10.28 +/- 1.0%.

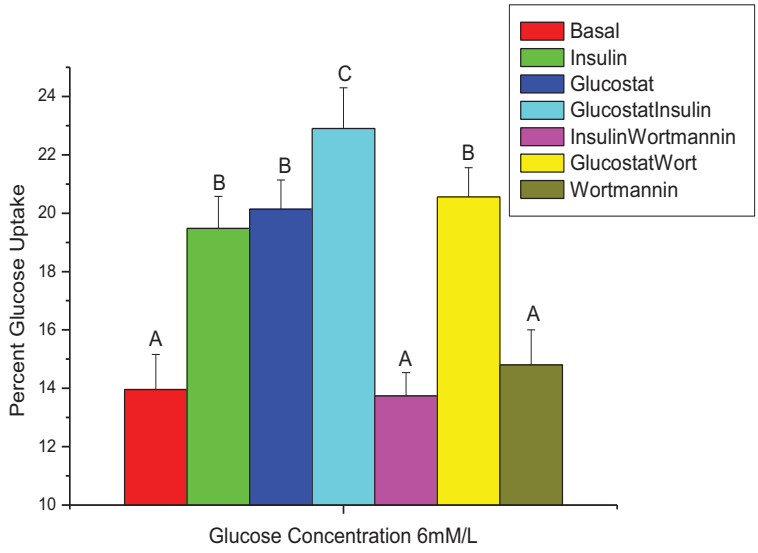



Figure 5.10: Glucose Uptake with the addition of Insulin and Glucostat and the effect of the addition of Wortmannin. Values expressed as means +/- S.E. Means without common letter are significantly different.

The glucose uptake when Glucostat, Insulin and Wortmannin were added to the culture was 10.24 +/- 0.9%, which is (P>0.05) significant, while that of Wortmannin on its own was 7.4 +/- 1.2% which is not (P>0.05) significant (See Figure 5.10).

The Boiled Glucostat showed an (P>0.05) significant increased uptake of glucose of 17.61 +/- 1.1% over basal, while 9.45 nmol/L of insulin alone resulted in a (P>0.05) significant uptake of 14.2 +/- 0.9%. Ten μg/ml of Boiled Glucostat alone (P>0.05) significantly decreased the glucose uptake to 17.61 +/- 1.0%. When the maximum dosage of insulin was added with the 10 μg/ml Boiled Glucostat there was an (P>0.05) significant uptake of 15.5 +/- 0.9 %. On the addition of Wortmannin to the culture with insulin, the glucose uptake was (P>0.05) insignificant at 14.23 +/- 1.2%; and when the Wortmannin was added with the Boiled Glucostat culture the uptake, again, was (P>0.05) non-significant at 14.33 +/- 0.8%. The addition of Wortmannin on its own resulted in a (P>0.05) non-significant decrease in uptake of 14.2 +/- 1.1% (See Figure 5.11).

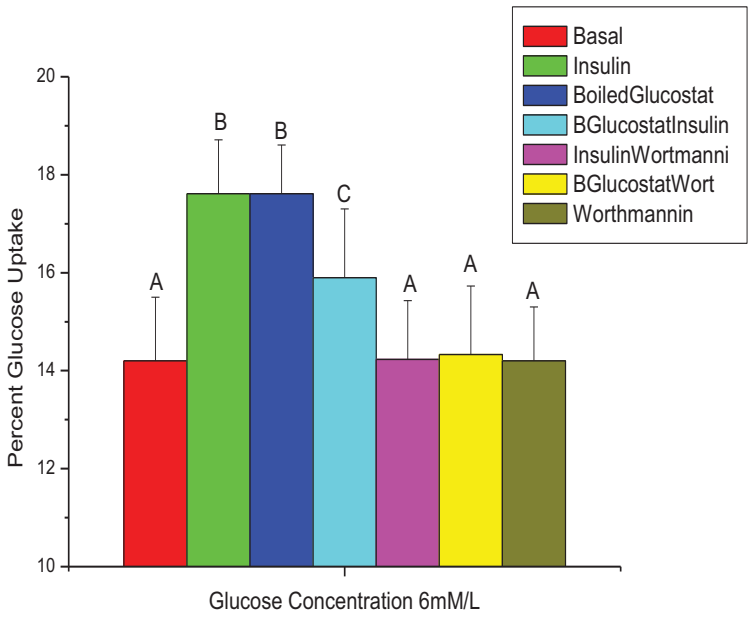



Figure 5.11: Glucose Uptake in mouse heart tissue in the presence of Insulin and Boiled Glucostat and the effect of the addition of Wortmannin. Values expressed as means +/- S.E. Means without common letter are significantly different.

Metformin is used to treat Type 2 Diabetes and so the interaction between it and Glucostat needed to be considered, and, therefore 100 mM Metformin was added with Glucostat in the presence of 8mM glucose to determine what effects the combination would have on glucose output in the mouse liver tissue. The basal uptake of glucose was 9.19 +/- 0.8% with Glucostat on its own ($P>0.05$) significantly increasing the uptake to 19.23 +/- 0.9%. With Metformin on its own there was ($P>0.05$) significantly increased uptake of 19.02 +/- 0.8%. When the Metformin and the Boiled Glucostat were combined there was a ($P>0.05$) significantly increased uptake of 21.27 +/- 0.9% (See Figure 5.12).

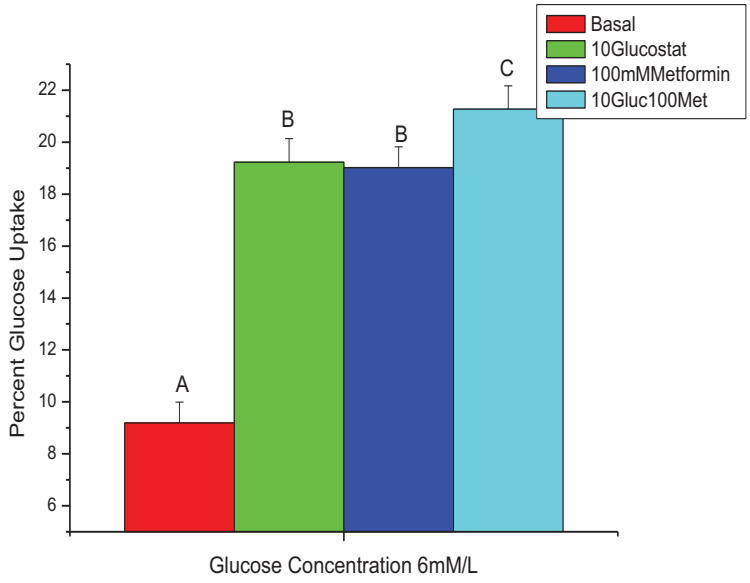



Figure 5.12: The effect of Glucostat with Metformin on heart tissue Culture. Values expressed as means +/- S.E. Means without common letter are significantly different.

The Boiled Glucostat was also added to 100 mM Metformin to determine if any interaction occurs. The basal uptake was 8.38 +/- 0.8% while the Boiled Glucostat ($P>0.05$) significant increased the uptake to a 16.18 +/- 1.0%. The Metformin on its own ($P>0.05$) significantly increased glucose uptake to 18.95 +/- 0.9%. However, the combination of Boiled Glucostat and Metformin ($P>0.05$) significantly increased the glucose uptake to 21.63 +/- 1.0% (See Figure 5.13).

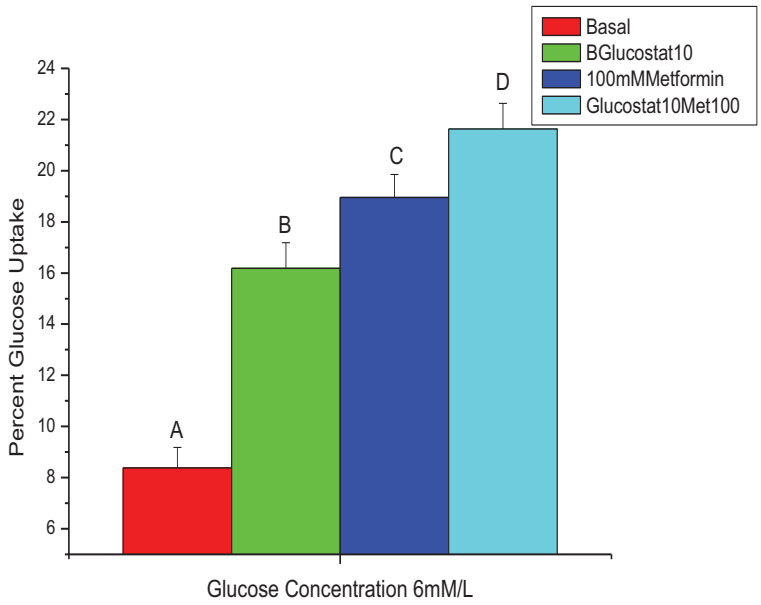



Figure 5.13: The effect of Boiled Glucostat (10 mins) with Metformin on heart tissue Culture. Values expressed as means +/- S.E. Means without common letter are significantly different.

In the liver tissue cultures when Metformin at a concentration of 100 mM was added with 10 µg/ml Glucostat in the presence of 8mM glucose to determine what effects the combination would have on glucose output in the mouse liver tissue. The basal level of glucose output by the liver was 13.6 +/- 1.2%, while that of Glucostat alone was ($P>0.05$) significantly decreased to 7.13 +/- 1.4%. Metformin alone ($P>0.05$) significantly decreased the output to 10.38 +/- 1.3%. However, when 100 mM Metformin was combined with 10 µg/ml of Glucostat there was a ($P>0.05$) significantly decreased output of 4.77 +/- 1.3% (See Figure 5.14).

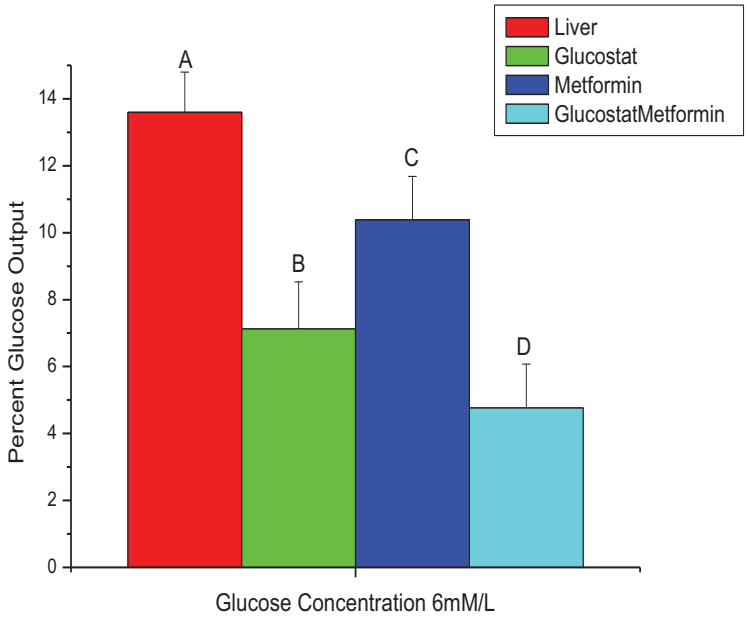



Figure 5.14: The effect of Glucostat and Metformin on liver Culture. Values expressed as means +/- S.E. Means without common letter are significantly different.

In the liver tissue culture when 100mM Metformin was added with 10 µg/ml Boiled Glucostat in the presence of 8 mM glucose to determine what effects the combination would have on glucose output in the mouse liver tissue. The basal level of glucose output by the liver was 13.7 +/- 1.2% while that of Boiled Glucostat alone was (P>0.05) significantly decreased to7.12 +/- 1.3%. Metformin alone (P>0.05) significantly decreased the output to 10.34 +/- 1%. However, when 100 mM Metformin was combined with 10 µg/ml of Boiled Glucostat there was a (P>0.05) significantly decreased output of 4.55 +/- 1.1% (See Figure 5.15).

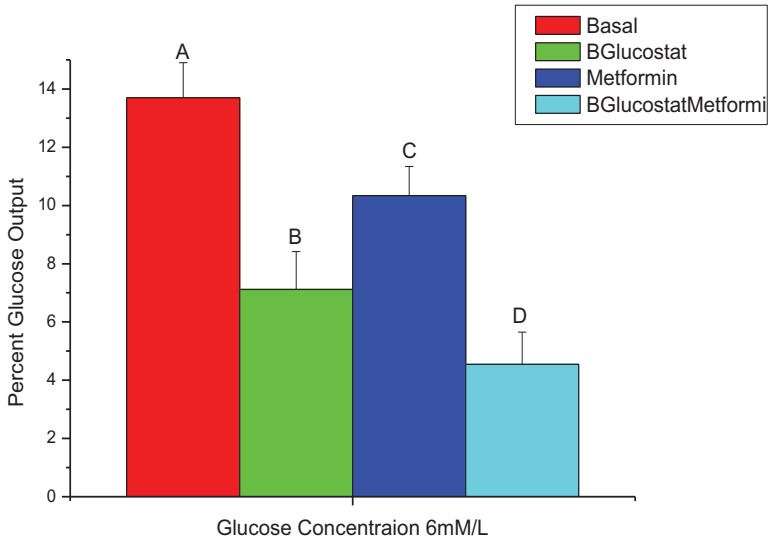
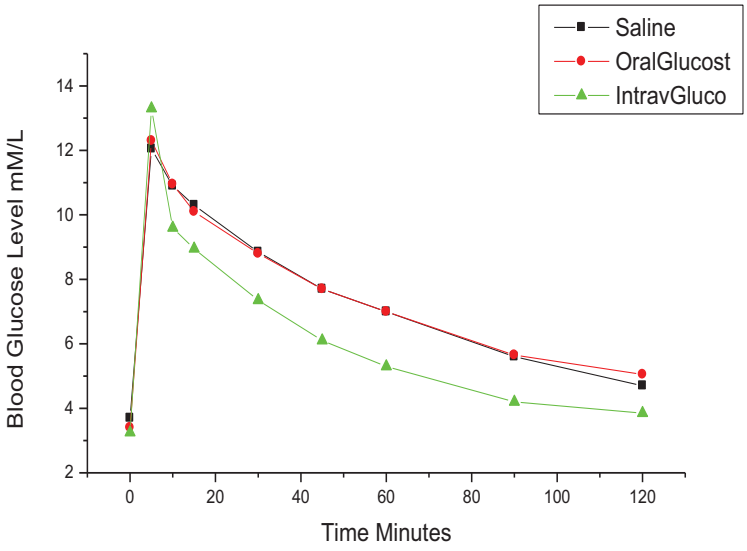



Figure 5.15: The effect of Boiled Glucostat with Metformin on liver Culture. Values expressed as means +/- S.E. Means without common letter are significantly different.

## 5.3.2. The Effect of GLUCOSAT on Glucose Clearance Rates in Sheep.

In order to determine if Glucostat given orally or intravenously affected the plasma clearance rate of glucose in the short term, a Glucose Tolerance Test was performed on treated and untreated merino wethers. The 2 wethers given Glucostat orally did not show any difference in clearance rates of glucose to those of the 2 wethers that were given saline (See Graph 5:16). The 2 wethers given the Glucostat intravenously however, did show a discrepancy. One of these wethers showed a glucose clearance rate the same as the wethers given saline, while the second of the wethers given Glucostat intravenously showed a much greater clearance rate of glucose (See Graph 5:16)




Graph 5:16 Glucose Clearance in Sheep treated with Glucostat. Mean of two sheep without treatment, treated orally with Glucostat and treated with intravenous Glucostat.

## 5.4 DISCUSSION

There have been numerous studies undertaken on *Trichosanthes kirilowii*, *Panax ginseng*, and *Stevia rebaudiana* and some their active constituents, along with a small number on *Polygonatum sibiricum* and *Dioscorea oppostia*. However, there has been no study undertaken to examine the effect of an aqueous extract of a combination of these herbs, available commercially in Australia and known as Glucostat, on muscle and liver tissue, both of which are important in glucose homeostasis. This study was undertaken to look at the effectiveness of an aqueous extract of Glucostat on Swiss mouse heart and liver.

Insulin resistance is a major indicator of Type 2 Diabetes and may be present long before the FBGls show any hyperglycaemic effect as the increased

insulin output will often counteract this problem. Insulin resistance is due to the impairment of the insulin signalling cascades, glucose transport and translocation of GLUT 4 in particular (Kellerer *et. al.,* 1999). Gluconeogenesis also plays a major role in Type 2 Diabetes because it becomes uncontrolled and is not switched off when normal FBGLs are reached (DeFronzo, 1997). The current management of diabetes looks at the insulin action and mimicking its action in order to decrease BGLs, both by increasing the uptake of glucose by tissues throughout the body and also decreasing gluconeogenesis. Thus, this study examined the effect of Glucostat in increasing glucose uptake by mouse heart muscle and also on liver tissue to decrease gluconeogenesis.

This study showed that there was a significant increased uptake of glucose into heart tissue culture and a decrease in glucose output from the liver tissue when 5 μg/ml and 10 μg/ml of Glucostat was added to the culture and that the increase was equivalent to that of 100 mM Metformin. However, it was noted that with increased concentrations of the Glucostat there was a slight decrease in uptake and increase in output which may have been due to the toxicity of the extract at higher concentrations as a crude extract was being used.

There was a decrease in the glucose uptake by the heart muscle and increased output by the liver tissue when using the boiled aqueous extract at lower concentrations; however, at 10 μg/ml it was approximately the same as that of the Glucostat and at 20 μg/ml it resulted in a higher uptake by the heart

tissue and a decreased output of glucose by the liver tissue. This suggests that there are at least two, but may be more active ingredients, involved in these results. It appeared that heat deactivated one or more of the active ingredients; however, there were also active ingredients that were not affected by boiling for 10 minutes. The active ingredient/s that were not affected by heat were only effective at higher concentrations, while the whole Glucostat appeared to be effective at lower concentrations suggesting that the active ingredient/s were affected by the boiling process.

A diethyl ether extraction was carried out to look at what type of chemicals might be involved in the increased uptake of glucose by the heart muscle tissue. The study showed that there was a slightly higher uptake of glucose in the heart tissue with both the aqueous phase and the solvent phase of the extract over that of the whole Glucostat. The glucose output from the liver was less with the aqueous phase of the extract then that of the solvent phase which was approximately the same as the whole Glucostat. The aqueous phase of the Boiled Glucostat showed approximately the same increase in uptake of glucose by the heart tissue, however, a slightly increased uptake when the solvent phase was used. In the liver the glucose output was approximately the same for aqueous phase and solvent phase of the extract, and both produced a lower output then the pure Boiled Glucostat, indicating that there are a number of different active ingredients within the combination of herbs. Further studies would need to be carried out to isolate the compound/s that are bringing about

the increase in glucose uptake by the mouse heart tissue and decreased output by the liver tissue in the both the unBoiled Glucostat and Boiled Glucostat.

The differences in glucose uptake by the mouse heart tissue and deceased output by the liver over basal, at different concentrations of glucose indicates that Glucostat has little or no effect when the glucose level is within normal range, but increases the uptake and decreases the output when glucose levels are high. This indicates that the taking of Glucostat will not lead to hypoglycaemia, coma and possible death. Very high levels of glucose were not tested as it was out of the scope of this study and therefore the effect on higher glucose levels needs further studies to determine the Glucostat's effectiveness at these concentrations.

A preliminary study looking at the mechanism of action, a maximum dose response of insulin (69.45 nmol/L) and also Wortmannin, which blocks the insulin pathway at the P13-K level by inhibiting P13-kinase (Hausdorf et al, 1999) was added to the Glucostat and glucose uptake measured. The enhanced uptake when Glucostat and insulin are combined indicates that the Glucostat may be acting via an insulin-mediated peripheral glucose pathway. However, it was noted that the addition of Wortmannin has no effect on the glucose uptake, suggesting that the Glucostat is entering the insulin pathway below the P13-kinase level. Insulin and Boiled Glucostat resulted in approximately the same glucose uptake by the heart muscle, however, when insulin was added to the Boiled Glucosat there was an inhibitory effect, suggesting an antagonistic effect.

When the Wortmannin was added to the Boiled Glucostat it was noted that the uptake is inhibited, suggesting that the active constituents of the Boiled Glucostat entered the insulin pathway above the PI3-kinase level.

Today many patients who have Type 2 Diabetes are prescribed Metformin in the early stages of the disease progression and therefore, the interaction between Glucostat and Metformin was investigated. On their own the Glucostat and Metformin resulted in approximately the same increased glucose uptake by the heart tissue and decreased glucose output by the liver. However, when Glucostat and Metformin were added to the heart or liver tissue cultures together there was an increased uptake and a decreased output of glucose compared to either of them alone. On their own the Boiled Glucostat resulted in a decreased uptake compared to the Metformin, however, in the liver the Boiled Glucostat resulted in a greater decrease of glucose output than the Metformin. However, when Glucostat and Metformin were added to the heart or liver tissue cultures together there was an increased uptake and a decreased output of glucose compared to either of them alone.

The single sheep experiment showed that Glucostat taken orally did not have any effect on glucose clearance compared to the treatment with saline. Glucostat given intravenously, however, showed a greater clearance rate than those treated with saline. Further testing over a longer period of time needs to be performed to determine the effect of intravenous injection of Glucostat on glucose clearance. The Health World Limited Technical Data Sheet for

Glucosat (2001) suggests that there is an increase in glucose clearance in humans; however, this could not be confirmed in sheep.

## 5.5 CONCLUSIONS

An aqueous extract of Glucostat increases the uptake of glucose in mice heart tissue culture and decrease gluconeogenesis from the mouse liver in a dose-dependent manner, with the optimum concentration being 10 μg/ml. It also may be slightly toxic at higher levels, with a decrease in glucose uptake by heart muscle tissue culture and increased gluconeogenesis in liver tissue culture over that of the optimum concentration. The toxicity may be due to the crude extract that was used or that some active ingredient comes into effect at the higher concentrations so there could be seen, both this toxic effect to the heart and liver tissue as well as an increased uptake of glucose. Boiling of the Glucostat decreases its ability to increase the uptake of glucose in the mouse heart tissue or decrease the glucose output in liver tissue culture at lower concentrations; however, at higher concentrations it increases the uptake and output over that of the unBoiled Glucostat. The diethyl ether solvent extract showed that both the aqueous phases and solvent phases of both the Glucostat and Boiled Glucostat had active ingredients suggesting that there were a number of different types of active constituents in the Glucostat. The addition of insulin and Wortmannin indicates that the Glucostat is acting via the insulin pathway, with some of its active constituents entering below the PI3K level, while some

of its active constituents entered above this level. There was a synergistic reaction when Glucostat and Boiled Glucostat were added with Metformin to the heart and liver tissue cultures. This indicates they could possibly be used in conjunction with each other; however, much more research needs to be carried out to determine if there are any interactions and increased efficacy and efficiency.

Glucostat given orally does not appear to have any effect on glucose clearance in sheep, however, there is an increase in glucose clearance rate when given intravenously, supporting the *in vitro* studies.

In summary, Glucostat and Boiled Glucostat both appear to increase glucose uptake in the mouse heart muscle and decrease gluconeogenesis in the liver. There appeared to be several active ingredients, however, isolation studies need to be undertaken to determine exactly what they are. The active ingredients appeared to be working through the insulin pathway, with some acting above the PI3K level and others below, and there is a synergistic effect on the addition of Metformin.

There needs to be further studies carried out to determine the Glucostat's active ingredient/s and their mechanism of action.

# 6. CLINICAL TRIALS

## 6.1 INTRODUCTION

The current treatment for Type 2 Diabetes is with oral hypoglycaemic medications, such as Metformin, and at advance stages, insulin. These medications can all result in various side effects including, abdominal discomfort, cholestatic jaundice, dermatological reactions, agranulocytosis, anorexia and metallic taste (Rang & Dale, 1991) and even death, and although preventative they are not a cure.

Throughout the world many different cultures use different herbal medicines for the treatment of Type 2 Diabetes and the symptoms associated with it. This has led to an increasing interest in the use of these traditional systems in search for a cure. However, little if any scientific evidence is available to show the efficacy and effectiveness as well as safety of these herbal medicines.

Ayurveda, a traditional Indian Medical system, advocates a wide range of herbal medicines either singly or in combination. This system was described in scripts written in the time of *Charaka* and *Sushrata,* during the 6$^{th}$ Century B.C., and advocates more than 100 different plant species (Grover & Vats, 2001). *Curcuma longa* Linn. has been used in the traditional Ayurevda Medical system as an anti-inflammatory and antimicrobial and for many other curative properties (Ammon and Wahl, 1991). The main colouring agent of Turmeric is curcumin and it has been shown to have a wide range of effects, including antihepatotoxic (Shishodia, *et. al.,* 2005), anti-inflammatory (Srimal, 1997),

hypocholesterolemic (SubbaRao *et. al.,* 1970; Patil & Srinivasan, 1971), choleretic and hydrocholagocic (Ramprasad & Sirsi, 1956), hypoglycaemic (Arun & Nalini, 2002), antioxidant (Quiles *et. al.,* 1998; Asai *et. al.,* 1999; Bengmark, 2006), anti-cancer (Huang *et. al.,* 1994; Kuo *et. al.,* 1996), antimicrobial (Gupta and Ravishankar, 2005), inhibits platelet-derived growth factor and has wound healing abilities (Kundu, *et. al.,* 2005).

Traditional Chinese Medicine, based on Taoist philosophy, proposes that the human body contains *yin, yang* and Five Elements (metal, wood, water, fire and earth), and that a perfect balance amongst all of these within in the body is required for perfect health (Li *et. al.,* 2004). If there is a imbalance amongst these from cold, heat, emotions or other factors then disease results. *Q*i (air) and blood serve as a communication channel between *Yin* and *Yang* and among the 5 elements. The primary aim of any Traditional Chinese Medicine is to restore the balance and replenish *Qi* or blood (Tang, 2006). Obesity and Type 2 Diabetes are a result of *Yin* deficiency with dryness-heat and needs to be treated by replenishing *Yin* (fluid) and evacuating fire (heat) from the body and is attributed to yin-deficiency diathesis, improper diet, emotional disorders, overstrain and excessive sexual activities (Li *et. al.,* 2004). Traditional Chinese Medicine is holistic with an emphasis on whole body care and then removal of the symptoms. Compound recipes are often used in Traditional Chinese Medicine, in that every herb provides a specialist function, as well as, form an integrated function for the treatment of disease, with the suggestion that a single

herb contains many ingredients but they cannot play the role of many herbs combined together (Li *et. al.,* 2004). Each of the herbs found within Glucostat act according to Traditional Chinese Medicine by either retoring *Qi,* or replenishing *Yin* or both, for example, Ginseng is used to replenish *Qi* and restore *Yin.*

Glucostat is a combination of the Chinese herbs, including *Trichosanthes kirilowii* (1900 mg), *Polygonatum sibiricum* (714.5 mg), *Dioscorea oppostia* (286 mg), *Panax ginseng* (95 mg), and *Stevia rebaudiana* (47.6 mg) per capsule. Apart from some evidence for the effectiveness of single herbs there is no evidence of the efficacy or efficiency of the combination in lowering of BGL in humans (see Chapter 5 of this thesis for discussion).

There have been several studies with the use of ethanolic extracts of Turmeric and its active ingredient, curcumin (Nishiyama et. al,2005) in the management of Type 2 Diabetes in mice (Arun & Nalini, 2002; Kuroda *et. al.,* 2005); however, there have been no studies looking at the hypoglycaemic effect in humans with the use of an aqueous solution. There have also been several studies on *Trichosanthes kirilowii*, *Panax ginseng,* and *Stevia rebaudiana,* along with a few on *Polygonatum sibiricum* and *Dioscorea oppostia;* however, there have been none on the anti-diabetic effect of the combination as found in Glucostat. Therefore, this study looks at the effects of 500 mg powdered Turmeric root dissolved in hot milk, taken twice per day and 1 gm Glucostat 3 times per day over a 3 month period in humans with a FBG >5.

## 6.2. MATERIALS AND METHODS

Plant extraction, human ethics approval, clinical trial and statistical analysis have been described in Chapter 2 of this thesis.

### 6.2.2. TURMERIC STUDY

Ten individuals, 3 males and 7 females, who had a FBG greater than 5 participated in the study. The ages ranged from 28-75 and BMI ranged from within normal to morbid obesity, while exercise ranged from low to fairly high. Eight had a history of Type 1 and/or Type 2 Diabetes in the Family. Seven were overweight to obese while 3 were within normal weight limits. Three did not exercise on a regular basis, while 7 of the patients did varying amounts of both aerobic and resistance exercise on a regular basis. Nine out of the 10 were on medication, with 5 taking medications for diabetes, 4 on varying amount of Metformin and 1 on Insulin. Other medications were for pain relief, anti-inflammatory which was stopped during the trial period, epilepsy, cholesterol, blood pressure and depression. Eight out of the 10 suffered moderate to severe arthritis, while only 3 had any history of cardiovascular disease, usually associated with high blood pressure and cholesterol. Fasting blood glucose levels ranged from 5.4 to 9.2 mM at the commencement of the study. In order to determine the effect of Turmeric on FBG levels, patients were asked to take daily readings with their individual hand held monitors and record the results.

### 6.2.3. GLUCOSTAT TRIAL

Eighteen individuals, 6 males and 12 females, who had a FBG greater than 5 participated in the study. The ages ranged from 28-75 and BMI ranged from within normal to morbid obesity, while exercise ranged from low to fairly high. Twelve had a history of Type 1 and/or Type 2 Diabetes in the Family. Fourteen were overweight to obese while 4 within normal weight limits. Four did not exercise on a regular basis, while 14 did varying amounts of both aerobic and resistance exercise on a regular basis. Ten of the 18 patients were also able to provide $HbA_{1c}$ readings before and after taking the medication. Seventeen out of the 18 were on medication, with 5 taking medications for diabetes and 4 on varying amount of Metformin. Other medications were for pain relief, anti-inflammatory, epilepsy, cholesterol, blood pressure and depression. Twelve out of the 18 suffered moderate to severe arthritis, while 15 had a history of cardiovascular disease, usually associated with high blood pressure and cholesterol. Fasting blood glucose levels commenced at 5.4 and went as high as 11.9 nmol/L. All stated that diet, as prescribed by their doctors/dieticians and exercise did not change throughout the trial period except when illness was present and exercise levels would drop for a short period of time.

## 6.3. RESULTS

### 6.3.1. TURMERIC TRIAL

Two patients did not respond to the Turmeric as can be seen in the patients RW and RM (Figures 6.1 and 6.2). Patient RW is a 54 year old female who was

diagnosed with diabetes 15 years ago, and is currently administering insulin tds. She also takes Diaformin, Karvea for blood pressure, Lipitor for cholesterol and Somac for reflux. Her BMI was in the obese range. RW did not show any significant effect as far as decrease in FBGls went, while taking the Turmeric; however, she did state that her diet was not as advised by her doctor/dietician and this may have contributed to the results. She did say, however, that she had commenced a walking program and she did feel better from that. Patient RM is a 75 year old female who was diagnosed with diabetes 6 years ago. She is not able to undertake much exercise due to surgical intervention and was taking Metformin and Lipex, and has a mother who suffered from diabetes. She did not suffer from any known arthritic condition. Her cholesterol was 5.4 and her BMI was within the low side of normal. Throughout the duration of the study her FBGL readings stayed similar to the pre and post study readings.

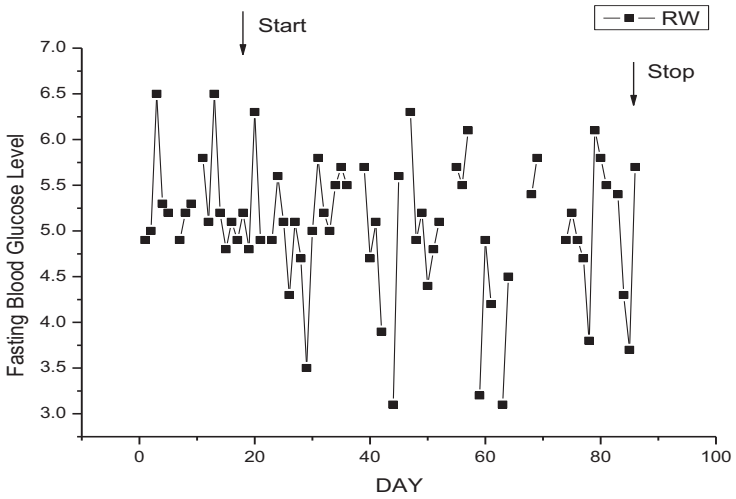



Figure 6.1: Fasting blood glucose levels before and during treatment with Turmeric. Arrows indicate the commencement and finishing days of the Turmeric.

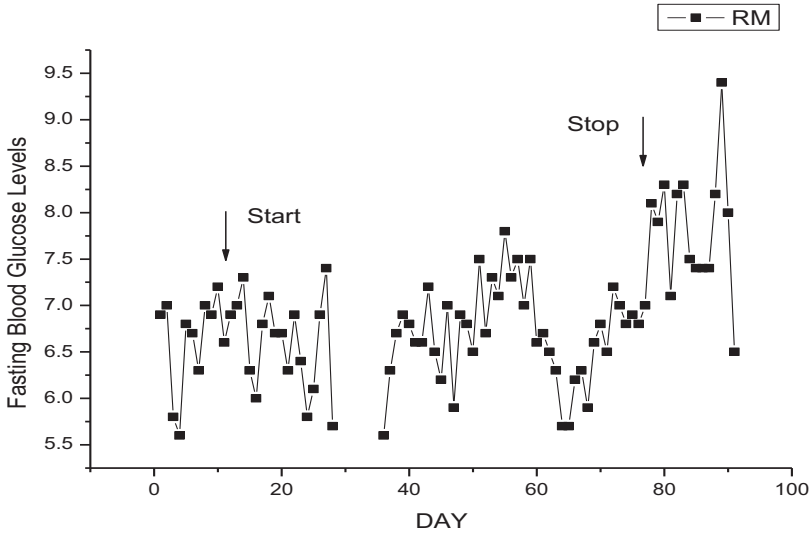



Figure 6.2: Fasting blood glucose levels before, during and after treatment with Turmeric. Arrows indicate the commencement and finishing days of the Turmeric.

Eight of the 10 patients responded to the treatment with Turmeric an example can be seen in Figure 6.3 with patient MC3, a 54 year old female who suffers from severe arthritic conditions and had very limited ability to undertake any exercise. She takes pain medication for severe, chronic pain as well as an

anti-epilytic for a condition known as Reflex Sympathetic Dystrophy, a hypotensive medication for slightly raised blood pressure and thyroxine for a slightly low functioning thyroid. She also has a history of diabetes on both sides of her family. During the period of the study the pain levels and swelling in joints decreased; however, exercises were still limited. Her FBGls decreased throughout the period of the study and when off the treatment they increased again to a level higher than before the treatment period. The FBGls decreased again when back on treatment. It can be seen that there was a gradual linear decrease in fasting blood glucose levels while on the Turmeric treatment. Upon cessation of the treatment the FBGls rose quickly after a short period of time, to a higher level than at the commencement of the study. On recommencement of the treatment with Turmeric FBGLs again decreased linearly.

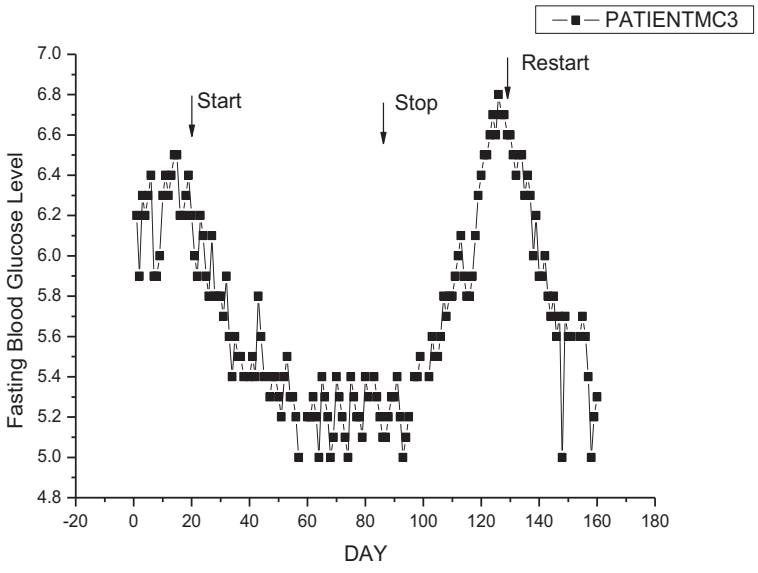



Figure 6.3: Fasting blood glucose levels before, during and after treatment with Turmeric. Arrows indicate the commencement and finishing days of the Turmeric.

The mean for the fasting blood glucose levels at the commencement of the study was 6.43 +/- 0.09 nmol/L. The highest level was 9.8 nmol/L and the lowest 4.8 nmol/L (a reading that was lower than normal for this patient which was very variable but generally in the range of 5.4 – 5.9 nmol/L). After the treatment period the mean dropped (P<0.001) significantly to 5.63 +/- 0.19 nmol/L. The means after the stopping of the treatment (P<0.001) significantly rose to 6.47 +/-0.24 nmol/L. For those that went back onto the treatment the mean fasting blood glucose level dropped (P<0.001) significantly to 5.68 +/- 0.19 nmol/L. There was a (P<0.001) significant decrease in FBGls of 12.4% or 0.8 +/- 0.17 nmol/L (Figure 6.4).

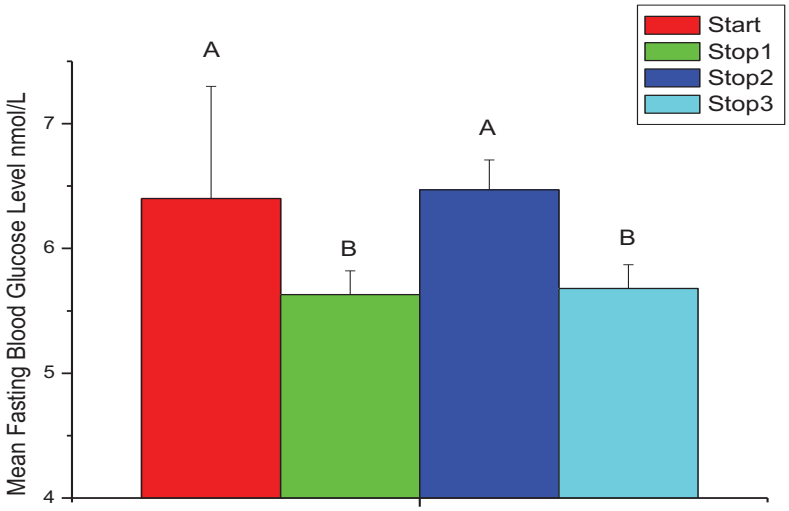



Figure 6.4: Mean fasting blood glucose levels of patients being treated with Turmeric. Start = pre-treatment FBGLs, Stop1 = FBGLs during treatment, Stop 2 = FBGLs after stopping treatment and Stop 3 = FBGLS after recommencing of treatment with Turmeric (n=10). Means with the same letters are not significantly different.

### 6.3.2 GLUCOSTAT TRIAL

In order to determine the effect of Glucostat on FBG levels, patients were asked to take daily readings with their individual hand held monitors and record the results. Summaries of 3 typical patients are shown in Figures 6.5 to 6.7.

One patient did not respond to the Glucostat treatment, as can be seen in the example of patient CG01 (Figure 6.5) This patient's FBGLs oscillated around their commencing value without any decline being shown over the 3 month period.

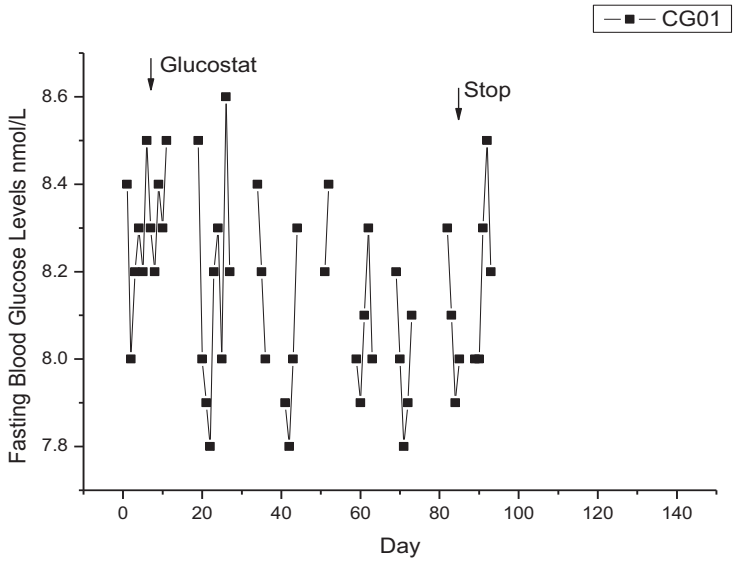



Figure 6.5: Fasting Blood Glucose Levels before, during and after treatment with Glucostat. Arrows indicate the commencement and finishing days of treatment.

Patient MC03 results show a significant gradual linear decrease in FBG levels while on the Glucostat treatment. Upon cessation of the Glucostat the FBG levels rose linearly again (Figure 6.6).

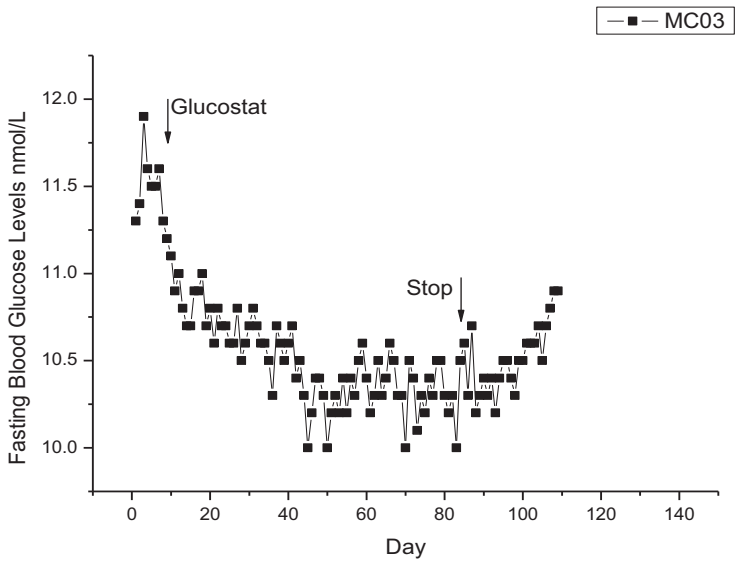



Figure 6.6: Fasting Blood Glucose Levels before, during and after treatment with Glucostat. Arrows indicate the commencement and finishing days of treatment.

Results for patient MC02 FBG levels decreased linearly after the commencement of the treatment and then upon stopping the treatment, the levels once again increased rapidly to original levels, However, after the recommencement of the Glucostat the FBG levels decreased linearly again to a lower level and at a much quicker rate then before. It can also been seen that the FBG levels plateaued out in the first period of treatment, while in the second period of this plateau was at a lower level (Figure 6.7).

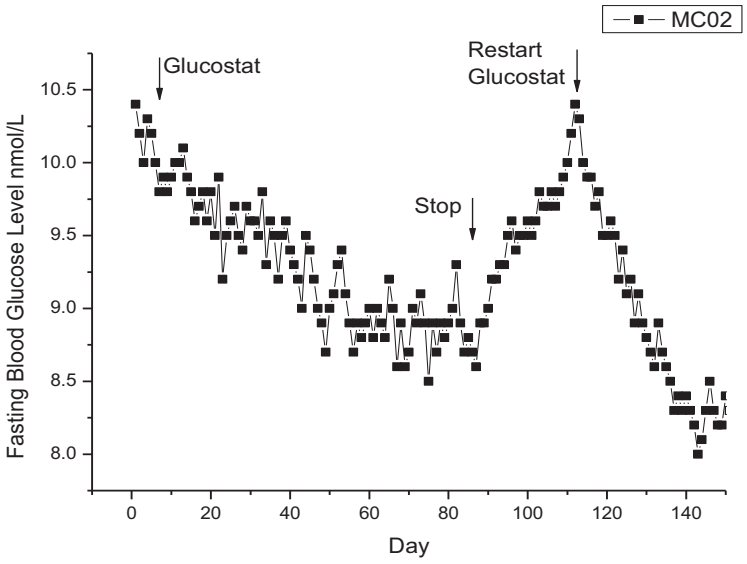



Figure 6.7: Fasting Blood Glucose Levels before, during and after treatment with Glucostat. Arrows indicate the commencement and finishing days of treatment.

The mean for the fasting blood glucose levels at the commencement of the study was 8.5 +/- 0.13 nmol/L. The highest level was 13.7 nmol/L and the lowest 5.8 nmol/L. After the treatment period the mean dropped ($P<0.001$) significantly to 7.44 +/- 0.0.17 nmol/L. The means after the stopping of the treatment rose ($P<0.001$) significantly to 8.66 +/- 0.21 nmol/L. For those that went back onto the treatment the mean fasting blood glucose level dropped ($P<0.001$) significantly to 6.67 +/- 0.2 nmol/L. There was a ($P<0.001$) significant decrease in FBGls of 12.47% or 1.06 +/- 0.14 nmol/L for all patients during the first treatment period (Figure 6.8). For those who recommenced the treatment, there was a ($P<0.001$) significant decrease of FBGLs of 22.9% or 1.99 +/- 0.21 nmol/L (Figure 6.8).

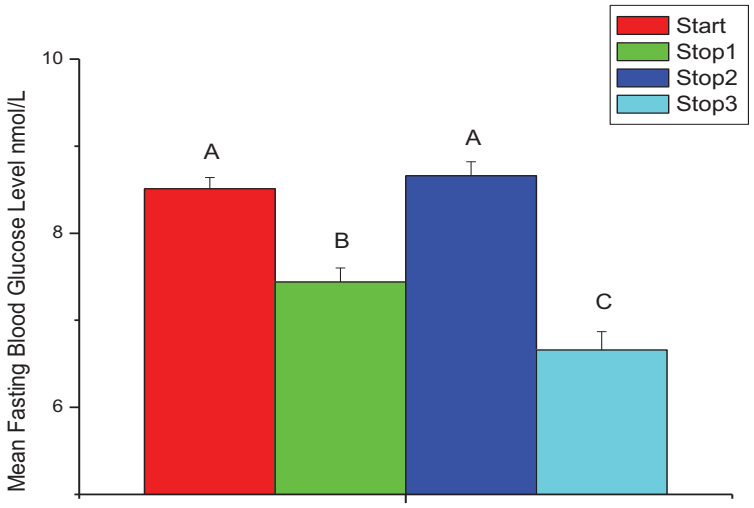



Figure 6.8: Mean fasting blood glucose levels of patients being treated with Turmeric. Start = pre-treatment FBGLs, Stop1 = FBGLs during treatment, Stop 2 = FBGLs after stopping treatment and Stop 3 = FBGLS after recommencing of treatment with Glucostat (n=18). Values expressed as means +/- S.E. Means without common letter are significantly different.

## 6. 4. DISCUSSION

Type 2 Diabetes is a chronic, slowly progressive disease with an inherited and/or environmental origin, with an increase in blood glucose concentration leading to the damage of many body systems, especially the blood vessels and nerves, resulting in retinopathy, nephropathy, neuropathy and heart disease and eventually to death. The prevalence of Type 2 Diabetes is increasing rapidly worldwide, primarily due to the increase of sedentary lifestyles and obesity (Uusitupa, 2002), with an estimated 177 million people suffering from it worldwide in 2002 (Dunstan *et. al.,* 2002; World Health Organization, 2002;) and this figure is expected to reach at least 324 million by the year 2025, or approximately 5.5% of the adult population (Meisinger *et. al.,* 2006a). In

Australia alone, there are 854,325 individuals that have been diagnosed with Type 2 Diabetes and registered with the National Diabetes Register, being 3.97% of the total population (Deed, 2009). It is estimated that another 400,000 who are not registered, are either pre-diabetic or do not realise that they have diabetes; this being one of the highest recorded prevalence's in the developed world. It has also been suggested that the average Australian has about a 1 in 14 chance of developing Type 2 Diabetes during their lifetime, and if impaired fasting glucose and impaired glucose tolerance are included, than approximately 1 in 4 Australians would be classified as having a glucose uptake disorder (Dunstan *et. al.,* 2002). The magnitude of the problem is enormous, with billions of dollars being spent annually by most western nations on the treatment of this disease and its complications. In developed countries Type 2 Diabetes claims about 9% of the National Health Budget (World Health Organisation, 2002) and in many countries it may account for 10% or more of the total health budget (Roglic *et. al.,* 2005).

Today there are several medications used to ameliorate the effects of diabetes and its complications by controlling blood glucose levels (BGL). These include oral hyperglycaemic drugs such as the sulfonylureas, biguanides and thiazolidinediones, and insulin which is used in the end stages of Type 2 Diabetes. Although the effects of the hypoglycemic drugs appear to be sufficient and BGL are normalised initially, they still tend not to stop the micro-vascular effects of the disease (Moss *et. al.,* 1994) and their effectiveness

appears to diminish overtime, therefore, an increased amount of a drug, or combination of drugs, must be taken to have the same effect (Cook *et. al.,* 2007). Thus the development of new and improved medications for prevention of Type 2 Diabetes will also assist in the real cost of this disease**,** as well as raising the well-being of those predisposed to the disease.

## 6.4.1. TURMERIC TRIAL

In any study it needs to be determined if the effects seen *in vitro* will be carried over to *in vivo* studies. As Turmeric could be considered safe for human consumption as it is used widely in cooking throughout the world as flavouring and colouring it was decided to run an introductory human trial. Patients, who had an FBGl > 5 were asked too participate in the study. In the Ayurvedic Medical System Turmeric would traditionally be taken with hot milk (Course material for Foundations in Ayurvedic Medicine, Metagenics) and therefore the participants were asked to take the Turmeric with a hot milk drink (tea, coffee, hot chocolate or hot low fat milk) twice per day.

Fasting blood glucose levels decreased in the patients who suffered from arthritis and were taking the Turmeric on a regular basis over the trial period. There was a gradual but ($P<0.001$) significant decline of 12.4% or 0.8 +/- 0.17nmol/L (Figure 6.4). On cessation of the treatment fasting blood glucose levels rose quickly and in the majority of cases went back to the same level if not higher then the original levels. In those patients who recommenced taking the herbs there was again a ($P<0.001$) significant decrease in the fasting blood

glucose levels to approximately the same level as when taking the treatment the first time.

There were 10 individuals who participated in the study and of those who showed improvement with the treatment of Turmeric, all had arthritis. Arthritis is a chronic inflammatory condition and now that there is a suggested link between inflammatory conditions and Type 2 Diabetes (Festa *et. al.,* 2002; Freeman *et. al.,* 2002; van Exel *et. al.,* 2002), consideration needs to be taken as to whether the results that were seen were due to the effect of Turmeric acting as an anti-inflammatory, which has been reported in the literature (Aggarwal and Sung, 2009). This may act by decreasing pain levels and stiffness in joints, thus, allowing the patient to undertake more exercise. This in itself would be of value in decreasing FBGl's or indirectly by weight loss. It may have also been due to a direct effect on the uptake of glucose in muscle tissue and the decrease of gluconeogenesis. All those with arthritis reported that their exercise regime did not change during the trial period, suggesting a more direct action of the Turmeric than just allowing greater amounts of exercise. The individual who was already on Metformin for the management of her Type2 Diabetes exhibited little, if any, change in her FBGl readings, although, when she stopped taking it there was a considerable increase over the introductory levels. The individual who was on insulin injections for her management, showed little difference at all but she did report that she she did not maintain her diet as set by the doctor/dietician, often foregoing her treatments and generally did not look after

herself. This individual did report that she was more able to undertake exercise and increased this a little due to decreased pain and swelling of joints; however, this still did not prompt her to actually increase her walking or other exercise. The patient on insulin stated that she felt more able to walk and undertake exercise after taking the Turmeric and started an exercise program with her friend; however, she reported often eating inappropriate foods such as chocolate and sweets which had a massive effect on her blood glucose levels. All patients with arthritic conditions stated that pain had decreased and one patient who had psoriasis said that this had decreased; however, they stated their exercise levels had not changed throughout the trial period.

Since the patients in the introductory clinical trial were asked to take the Turmeric in hot milk it is important to take into consideration if the hot milk itself may be influencing blood glucose levels rather then the Turmeric. Pittas and colleagues (2006) conducted a study looking at the milk intake of women over an 18 yr period and in particular, analysing the intake of calcium and Vitamin D. They showed that there was a significant inverse relationship between total Vitamin D and calcium intake and the risk of developing Type 2 Diabetes; the highest association being when supplements of the dairy products were taken. This was supported by Liu and colleagues (2006) who showed that women who consumed low fat dairy products had a decreased chance of contracting Type 2 diabetes than those who had high fat or no dairy products. It was suggested that this might have been due to those who consumed a low fat

dairy diet, also generally, had a healthier lifestyle rather than the direct effect on the Type 2 Diabetes. It was suggested that the decrease may be due to the dairy products resulting in an increase secretion of the insulinotropic amino acids and incretin along with the various components of dairy such as calcium, protein and lactose may increase satiety, reducing the risk of becoming overweight (Liu *et. al.,* 2006). However, this doesn't account for the decrease in FBGl's amongst the patients trialling the Turmeric as they all reported that their diet didn't change. They stated especially that their milk intake didn't alter except that some stated they didn't normally have hot milk but rather cold, so whether having hot milk affects the absorption of milk components and the secretion of incretin would need to be investigated further. The reason for taking of the Turmeric in hot milk was because this is the way traditionally used for taking Turmeric in the Ayruvedic Medical System. However, how the hot milk aids the absorption or the effectiveness of the Turmeric or its components is not understood at this stage and needs further investigation. It was also noted that one of the patients was lactose intolerant and took the Turmeric with Soya Milk. This particular individual did show a significant ($P<0.05$) improvement with the treatment and therefore the cow's milk may not play any role at all. Further studies will need to be carried out to ascertain the best way to take the Turmeric.

Further clinical trials will need to be undertaken to determine the effectiveness of Turmeric in a range of people, with and without chronic inflammatory diseases, as well as those who have a higher commencing FBGl as

it is noted, that those participating in this trial started with only slightly higher then normal levels.

## 6.4.2. GLUCOSTAT TRIAL

Fasting blood glucose levels decreased in the majority of patients who were taking the Traditional Chinese Herbs on a regular basis over the trial period. There was a gradual but ($P<0.001$) significant decline of 12.47 or 1.06 +/- 0.14 nmol/L during the first treatment period followed by a plateau in those patients who responded to the treatment. On cessation of the treatment, FBGLs rose quickly and in the majority of cases went back to the same level if not higher than the original levels. In those patients who recommenced taking the herbs, there was again a ($P<0.001$) significant decrease in the fasting blood glucose levels. However, it was noted that the decline was much steeper this time and the plateau occurred at a generally lower level then in the first period of treatment with a significant ($P<0.001$) decrease of FBGLs of 22.9% or 1.99 +/- 0.21 nmol/L. Research that has been undertaken on the individual herbs of *Trichosanthes kirilowii* (Ng *et. al.,* 1985; Hikino *et. al.,* 1989; Lu *et. al.,* 2001; Xu *et. al.,* 2001), Panax ginseng (Suzuki, 1989; Sotoniemi *et. al.,* 1995; Vuksan *et. al.,* 2001) and *Stevia rebaudiana* (Curie, 1986; White, 1994; Jeppesen *et. al.,* 2000), *Dioscorea opposita* (Hsu, J., *et. al.,* 2007), *Polygonatum sibiricum* (Bensky & Gamble 1993) along with the chromium (Roginiski & Mertz, 1969; Anderson, *et. al.,* 1997; Anderson, 1998;), have all been shown to have a blood

glucose lowering effect even though the mechanisms have not been elucidated, as yet.

## 6.5. CONCLUSIONS

### 6.5.1. TURMERIC TRIAL

The clinical trial suggests that Turmeric will aid in the decreasing of FBGl's of individuals who have a moderately high FBGL without having any effect on those with a low to normal level. This is particular in the case of those who have an arthritic condition, whether this is due to increasing the ability to exercise or a direct effect needs to be investigated further. There appeared to be little effect on those who were already on Metformin (n=3) or insulin (n=1); however, this being too small a number of participants to say this accurately. The effects may be due to the anti-inflammatory effect, allowing the individual to increase exercise; however, as the individuals in this study stated that exercise did not change during the study period there may also be a direct effect. Further controlled clinical trials would need to be carried out to determine if there is an increase in exercise or not.

There needs to be further studies carried out to determine Turmeric's active ingredient/s and the mechanism/s of action; along with Turmeric's effect on humans who have higher blood sugar levels and those who are already on different medications for the treatment of diabetes.

### 6.5.2. GLUCOSTAT TRIAL

This combination of Traditional Chinese Herbs and chromium appear to lower fasting blood glucose levels in the majority of patients, although the mechanism/s of action is not known at this stage and may include a combination of factors. These may include increase in insulin output, increased insulin responsiveness and glucose uptake. The relative importance of the individual herbs within the combination is also unclear at this stage and further studies need to be undertaken to determine this.

There needs to be further studies carried out to determine the active ingredient/s and the mechanism/s of action. Also further studies are needed to determine Glucostat's effect on humans who have higher blood sugar levels and are already on different medications for the treatment of diabetes. Only four patients were already taking Metformin in this study; however, this number is too low to determine the effect of the combination in the management of Type 2 Diabetes.

# 7. GENERAL DISCUSSION

## 7.1. INTODUCTION

The prevalence of Type 2 Diabetes is in epidemic portions worldwide, primarily due to the increase of sedentary lifestyles and obesity (Uusitupa, 2002), with an estimated 177 million people suffering from the disease worldwide in 2002 (Dunstan *et al.,* 2002; The World Health Organization, 2002). This figure is expected to reach at least 324 million by the year 2025, or approximately 5.5% of the adult population (Meisinger *et al.,* 2006). This increase will occur mainly in the developing countries, (170%), compared to developed counties who are expecting an increase of 42% (Dunstan *et al.,* 2002; Saudek, 2002). In 2002 it was estimated that Type 2 Diabetes, or its complications, was the fifth leading cause of death world wide (Roglic *et al.,* 2005). The magnitude of the problem is enormous with billions of dollars being spent annually by most western nations; and in developed countries 9-10% of the National Health Budget (World Health Organisation, 2002; Roglic *et al.,* 2005), is spent on Type 2 Diabetes.

The current treatments for Type 2 Diabetes include diet and lifestyle changes and oral antidiabetics or insulin injections. The current medications can maintain blood glucose levels within the normal physiological range for short periods of time; however, they do not appear to be able to maintain this, and more drugs or combinations of drugs are needed. New and novel treatments need to be found that mimic the action of insulin on tissues, does not bring on hypoglycaemia and also maintains its effect.

Many cultures have herbal medications that have been used for the treatment of Type 2 Diabetes or similar symptoms with more than 400 traditional herbal remedies having been recorded, however. only a few of these have received medical and scientific evaluation to determine effectiveness, efficacy, side effects and toxicity (Baily and Day, 1989). Since many plants have been used in various cultures for the management of Type 2 Diabetes the World Health Organisation has recommended that medical and scientific examinations of such plants be undertaken (World Health Organisation Expert Committee on Diabetes Mellitus, 1980), to determine their effectiveness, efficacy, side effects and toxicity. Therefore this study initially screened several culinary herbs and plants and then looked at the anti-diabetic effect of Turmeric and a combination of Chinese Herbs that were available commercially in Australia, where some studies had been performed on the individual herbs but not in combination.

Turmeric has had several studies carried out showing its beneficial effect on glucose levels, however none have been with an aqueous extract and no clinical trials have been noted (SureshBabu & Srinivasan, 1998; Arun & Nalini, 2002; Gupta and Ravishankar, S., 2005; Korado *et. al.,* 2005; Kundu, *et. al.,* 2005; Nishiyama *et. al.,* 2005; Shishodia, *et. al.,* 2005; Suryanarayna 2005; Yang, *et. al.,* 2005; Bengmark, S., 2006). The individual herbs in Glucostat, *Trichosanthes kirilowii*, *Polygonatum sibiricum*, *Dioscorea oppostia*, *Panax ginseng*, and *Stevia rebaudiana* have all been shown to have hypoglycaemic

abilities, however, the majority of the studies were carried out with an ethanolic extraction (Konno *et. al.,* 1984; Curie 1986; Hikino *et. al.,* 1989; Suzuki, 1989; Suzuki *et. al.,* 1991; Kato and Miura, 1993; Kato *et. al.,* 1994; Sotaniemi *et. al.,* 1995; Ohnishi *et. al.,* 1996; Malaisse *et. al.,* 1998; Attele *et. al.,* 1999; Onomura *et. al.,* 1999; Wallace, 1999; Jeppesen *et. al.,* 2000; Vuksan *et. al.,* 2000; Vuksan *et. al.,* 2001; Dey *et. al.,* 2003; Hsu *et. al.,* 2007), and also the herbs have never been tested as a combination as found in Glucostat except for one introductory clinical trial carried out (Collins and McFarlane, 2006). The present study was designed to look at the aqueous extracts of both the Turmeric and the Glucostat and begin to explore the mechanism of action as well as running introductory clinical trials.

## 7.2. SCREENING OF HERBS

An in-house developed *in-vitro* bio-assay method was employed in this study to screen the effects of treatments of an aqueous extract of the culinary herbs, *Laurus nobilis* tree (Bay Leaves), *Origanum vulgare* (Oregano), *Curcuma longa* Linn (Turmeric), *Ocimum basilicum* (Sweet Basil), *Lavandula officinalis* (English lavender), *Thymus vulgaris* (Thyme), *Cuminum cyminum* (Cumin), *Coriandrum sativum* (Coriander), *Metha piperita officinalis* (Peppermint), *Myristica fragrans* (Nutmeg),and *Eugenia caryophyllata* (Cloves), along with *Stevia rebaundia,* and *Gymnema sylvestre* and the Australian plants, *Eremophila longofolia* (Emu Bush), Grevilla species leaves and *Stenanethum scortecceni*

stems as well as Glucostat, a combination of traditional Chinese Herbs, on glucose uptake in mouse heart tissue. Many of the herbs screened were able to increase the uptake of glucose in mouse heart tissue (Chapter 3). However, it was decided to look further into Turmeric and Glucostat. Turmeric as it appeared to have the highest glucose uptake and as a culinary herb and used in the Ayruvedic Medical System should be safe to run a clinical trial. Glucostat as it was commercially available, and an introductory clinical trial had already been undertaken (Collins and McFarlane, 2006).

The pathogenesis of Type 2 Diabetes is largely unknown and very complex; however, the major components appear to be insulin resistance and β-cell dysfunction (Reviewed in Defronzo, 1992) as well increased gluconeogenesis. The current medications for Type 2 Diabetes increase insulin production, secretion and sensitivity, decrease gluconeogenesis, mimic insulin action, delay gastric emptying and glucose absorption or alter renal glucose handling. However, none have been able to prevent the progression of the disease and its many complications. with the loss of insulin production resulting in the need for exogenous insulin therapy. Therefore, research is needed to search for new and novel compounds that will increase glucose uptake, reduce gluconeogenesis and protect, preserve and increase β-cell mass leading to major advances in the management of Type 2 Diabetes. Hence, the current advancements of antidiabetic treatments are focused on plants that have been used for centuries by different cultures (World Health Organisation Expert Committee on Diabetes

Mellitus, 1980). The present work was carried out on plants that had potent effects on glucose uptake and gluconeogenesis. Of the two treatments that further study was carried out on (Turmeric and Glucostat); the effects have suggested single or multiple constituents that may act directly or indirectly on insulin pathways within the muscle cell and liver.

## 7.3 TUSSUE CULTURE STUDIES USING TURMERIC AND GLUCOSTAT

Both Turmeric and Glucostat showed a dose-dependent effect on glucose uptake by the mice heart tissue and output by the mice liver tissue only in hyperglycaemic conditions but not in normoglycemic, indicating that these treatments would not lead to hypoglycaemia under normal physiological states. The enhancement of glucose uptake activity of Turmeric and Glucostat in the presence of a saturated dose of insulin, suggests these extracts possibly act via insulin-mediated enhanced peripheral glucose uptake pathways, similar to Metformin, the well established antidiabetic drug (Meyer *et. al.,* 1967; Jackson *et. al.,* 1987; Bailey, 1988; Hother-Nielson *et. al.,* 1989; Galuska *et. al.,* 1994; Fisher *et. al.,* 1995). Interestingly, Wortmannin (a PI3-kinase inhibitor), inhibited the glucose uptake effects of Turmeric but not Glucostat, suggesting that Turmeric works on the classical P13-Kinase pathway, entering above the PI3-kinase level and Glucostat below this level.

Turmeric is often dry roasted when used as a culinary herb in cooking of such cuisines as curries so it was decided to test for the antidiabetic effect of this

as well as boiled aqueous extract. The dry roasting of the Turmeric before extraction and boiling of the extraction removed all activity of the Turmeric as far as increased glucose uptake by the mice heart tissue and decreased glucose output by the mice liver tissue. The aqueous phase of the diethyl ether extraction enhanced the glucose uptake in the mice heart tissue and decreased the output in the mice liver tissue, suggesting that there had been a purification or concentration of the active constituent/s by the extraction, while the solvent phase showed little change over basal levels. Curcumin, the chemical that has been shown to be effective in lowering glucose levels in previous studies (Hasmeda & Polya 1996; Arun & Nalini, 2002; Ikonomov *et. al.,* 2002; Korado *et. al.,* 2005; Nishiyama *et. al.,* 2005) would be found in the solvent phase of the extract indicating that there is a different compound that is bringing about the increased glucose uptake. Considering the loss of activity when the Turmeric solution was boiled or the Turmeric was dry roasted before the aqueous extract was made, suggests that the active component is a protein. Interestingly, the non-aqueous portion increased the uptake to a greater extent than that of the straight Turmeric, suggesting that there is an ingredient in the aqueous portion that may be inhibiting the uptake or that the more purified sample was more efficient. Previous studies have shown that Curcumin has an effect on decreasing BGLs; however, this study showed that the non-aqueous extract was producing a different active constituent which is having a similar effect, as the Curcumin would be found in the aqueous portion. Further studies would need to

be carried out to isolate this compound that brought about the increase in glucose uptake by the mice heart tissue.

Boiling of the Glucostat resulted in a different pattern than that of the Turmeric with both the original aqueous extract and the boiled aqueous extract resulting in increased glucose uptake mice heart tissue culture and decreased output by the mice liver tissue culture; however, at the lower concentrations the Boiled Glucostat showed less of an effect than the straight extract but at the higher concentrations the Boiled Glucostat was more effective. This suggests that there are a number of different active compounds.

## 7.4 CLINICAL TRIALS

Often drugs will work in cell culture and/or tissue culture but will not be effective in humans and/or animals due to absorption or other problems. Therefore it is necessary to trial all possible medications in animals and then humans before being able to say that they will be effective. In order to do this, it is also necessary to be sure there was no major side effects that could lead to serious illness, injury or even death, however, in this study since Turmeric is used daily in kitchens throughout the world and Glucostat was a commercially available product, we did not have such concerns about this especially if Glucostat, was taken as recommended by the manufacturer.

Individuals who had blood glucose levels greater than 5 were asked to participate in the study and it was shown that Turmeric was effective in ($P>0.001$) significantly decreasing FBGls by 12.4% or 0.8 +/- 0.17 nmol/L.

The major effect appeared to be in those who were suffering from the inflammatory condition of arthritis and this may have been due to the anti-inflammatory effect of Turmeric which allowed the individuals to increase exercise due to less pain; however, the participants stated that their exercise regime generally did not change throughout the study period so there appears to be a direct effect as well. In those suffering from arthritis there was a gradual decline in glucose concentration after a short period of starting the treatment and then a gradual rise back to commencing levels or slightly higher after going off the treatment. For those who went back onto the treatment, there was again a short delayed response and then a gradual decline back to the same level as when previously taking the herb. However, Glucostat was effective in all patients and although the effects took time to become apparent there was a ($P<0.001$) significant decrease in the concentration of glucose in the blood. It was noted that after ceasing the medication that the FBGL rose rapidly to the pre-treatment levels and in some cases higher, and with those who recommenced the treatment there was a more rapid decline in FBGLs than during the initial treatment period.

Both of the clinical trial for Turmeric and for Glucosat was very limited due to the small number of participants, though there was sufficient to give an indication that both of these treatments may be of benefit in the management of Type 2 Diabets. Another limitation of these trials was the inability to monitor the participants as far as diet and exercise levels. Even though participants

reported diet and exercise had not changed this was not validated. In order to determine the longer term effects there also needs to be a much longer term trial to verify if the treatments will be beneficial on a long term basis.

## 7.6 SUMMARY

In summary both Turmeric and Glucostat appear to be effective in decreasing glucose levels in tissue culture, and also in clinical trials where people have a low to moderate increase in FBGls over the recommended. However, there needs to be considerabley more research undertaken to isolate the active constituient/s and their mechanism/s of action. Further controlled clinical trials need to be undertaken with both Turmeric and Glucostat to determine the effectiveness in different groups of people, the best mode of administration of the medication and also to find if they will be effective in people who are already on medication such as Metformin and those who have a much higher blood glucose concentration.

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