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# PHCOG REV.: Invited Review
# Cell lines in Diabetes Research: A Review

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**ABSTRACT**
Animal models have been used extensively in diabetes research. Early studies used pancreatectomised dogs to confirm the central role of the pancreas in glucose homeostasis, culminating in the discovery and purification of insulin. Selective inbreeding has produced several strains of animal that are considered reasonable models of Type I diabetes, Type II diabetes and related phenotypes such as obesity and insulin resistance. Today, animal experimentation is contentious and subject to legal and ethical restrictions that vary throughout the world. This review gives an overview on the various cell lines used to evaluate the new chemical entities (NCEs) for their hypoglycemic effects. These cell lines are described with their origin/source, characteristic features, mechanism(s), advantages/disadvantages and applications in diabetes research in screening of NCEs for type I and type II diabetes. In addition, it especially describes the appropriate selection and usefulness of different cell line in preclinical testing of various NCEs for the treatment of diabetes.
**KEYWORDS**: Cell lines, diabetes research, type I diabetes, type II diabetes.

## INTRODUCTION

### *Rational behind the in vitro models used in diabetes research*

A range of *in vitro* and *in vivo* models are available to study potential antidiabetic activity in plant extracts. They are base on the primary need to control hyperglycemia in diabetic and the various means of achieving these goals. *In vitro* models may be used to screen randomly or ethnobotanically selected material for a specific activity that would result in lowering of blood glucose levels. Alternatively the models may be used to determine the mechanism of action of plant extract with traditional use and/or human *in vivo* data to support an antidiabetic effect.

It is relevant in the context of *in vitro* test for antidiabetic activity, to examine the source and fate of glucose in the body in the normal and diabetic states. Glucose is derived primarily from the digestion of dietary carbohydrates in the gastro intestinal tract from which it is absorbed in to the blood by passive and active mechanisms. In the fed state, a rise in blood glucose normally stimulates insulin secretion from the pancreas. This hormone initiates glucose uptake in to specific target tissue, primarily liver, muscle and fat cells (adipocytes). It promotes glucose oxidation and glycogen deposition in liver and muscle and the incorporation of glucose (as glycerol) in to triglycerides in adipocytes. These combined activities have the effect of lowering elevated plasma glucose resulting from the intake of a meal. In the fasted state, insulin and glucose level decrease. Glucose is then metabolized from glycogen stored in liver (glycogenolysis). Another important source of glucose in the fasted state is gluconeogenesis – the *de novo* formation of glucose from smaller, unusual precursor molecules. This occurs in the liver and to a lesser extent, kidney and is under the control of glucagons, a common counter hormone whose level rise as those of insulin fall and vice versa. When glucagons levels are high and those of insulin are low gluconeogenesis and glycogenolysis are stimulated and glucose enters the blood stream.

In diabetes insulin is absent (Type I diabetes) or insufficient (Type II diabetes). In Type II diabetes insulin target tissues are generally less responsible to insulin (insulin resistant) than normal. The fine balance between glucose uptake in to target organs and release of hepatic glucose is impaired, resulting in abnormal high fasting glucose levels as well as poor glucose tolerance following a meal.

From these, following mechanism has been proposed for an agent that could lower or control plasma glucose level.

- Inhibition of carbohydrate digesting enzymes, reducing the amount of rate of glucose release from the diet
- Impairment of glucose uptake from small intestine.
- Stimulation of insulin secretion from ß cells of pancreas.
- Insulinomimetic or insulin secreting activity at insulin target tissue i.e, liver, skeletal muscle or adipocyte.
- Antagonism of glucagons activity.

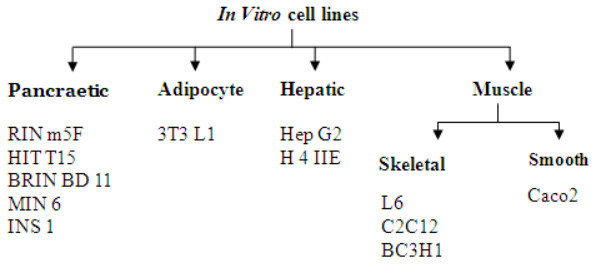


***Figure 1: Cell lines used in Diabetes Research***

**SCREENING FOR ANTIDIABETIC EFFECT IN CELL CULTURES**

***Pancreatic B-cell line*:**

***RIN m 5F***

This rat insulinoma cell cultures were initiated from a transplantable islet cell tumor, induced by high-dose x-irradiation in an inbred NEDH (New England Deaconess Hospital) rat. The tumor was maintained by serial transplantation in NEDH rats. Continuous cell lines were first derived either from rat transplants (Joslin group) or from nude mouse heterotransplants (NCI-VA group). These cell lines were named RIN-r and RIN-m, respectively. The RIN-m cell line was established from the fourth nude mouse heterotransplant. Cultures from earlier transplants resulted only in temporary growth of epithelioid cells (1) RIN-r cultures consisted of epithelioid cells without apparent fibroblastoid contamination (Fig. 1A); their appearance was similar to that of RIN-m cells (Fig. 1B). The population doubling time was approximately 52 hr in medium 199 with 10% fetal bovine serum.

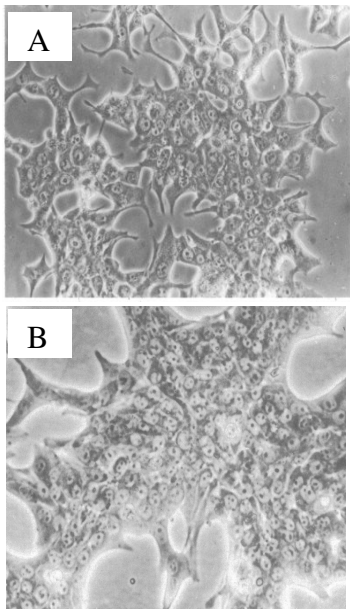


***Figure 2: Phase-contrast photomicrographs of RIN cells. The cells are epithelioid, and cytoplasmic processes extend from free cell surfaces.(A) RIN-r cells. (X 120); (B) RIN-m cells (X 210).***

This continuous culture of pancreatic islet cells provides a potentially valuable tool for diabetes research. The RIN cell lines offer important models to study the mechanisms and control of insulin and somatostatin secretion. They may also be used to identify hormone receptors and antigens on their surfaces and to determine the nutritional requirements for hormone secretion in defined media.The insulin-producing rat cell line RINm5F (2) has been found to respond to a variety of agents with appropriate changes in rates of insulin release (3). In RINm5F cells, GLP-1 (Glucagon-like peptide-1, a potent incretin hormone secreted from distal gut) increased the capacity and affinity of insulin binding in a time- and concentration-dependent manner (4). Evaluation of many synthetic compounds for their *in vitro* glucose dependent insulinotropic activity has been done usind RIN 5F cell based assay (5).

Latha et al, has reported insulin-secretagogue activity of an antidiabetic plant extract (*Scoparia dulcis*), its protective action against streptozotocin- mediated cytotoxicity and nitric oxide (NO) production in rat insulinoma cell line, RINm5F (6). Halban et al. studied glucose metabolism and insulin release in isolated rat islets and in an insulin-producing rat cell-line (RINm5F) and found that the changes in glucose metabolism may contribute towards the failure of glucose to stimulate insulin release from RINm5F cells (7). The RINm5F cell-line has been established as the first model in permanent culture known as expressing an alpha-2 adrenoceptor of the alpha-2D subtype and as a good system for studying *in vitro* the respective role of alpha-2 adrenoceptors and NAIBS (nonadrenergic idazoxan-binding sites )in the regulation of insulin secretion by beta cells (8).

***HIT-T15***

The availability of transplantable rat and hamster insulinomas has provided considerable impetus to studies of beta-cell physiology and molecular biology (9-11). Santerre et al. reported in 1981, a clonal hamster beta cell line (HIT) which was established by direct *in vitro* treatment of primary cultures of Syrian hamster pancreatic islet cells with ethyl methanesulfonate and transformation with simian virus. In this cell line cytoplasmic insulin was detected in all cells by indirect fluorescent antibody staining. HIT-T15 cells contained membrane-bound secretory granules, which were identical to secretory granules in normal hamster beta cells (12, 13).

HIT cell insulin secretion was stimulated by glucose, glucagon, and 3- isobutyl-l-methylxanthine. Insulin secretion at optimal glucose concentration (7.5 mM) is 2.4 milliunits per 10 cells per hr. Somatostatin and dexamethasone markedly inhibit HIT insulin secretion (14). The HIT cell line represents a unique *in vitro* system for studying beta cell metabolism and insulin biosynthesis. This cell line represents an unique model system with which to study beta cells. For example, it can provide unlimited material for biochemical studies of membrane receptors or mRNA processing. Qualitatively, HIT cells retain most, if not all, the differentiated functions characteristic of beta cells and can serve as a model system to study gene expression and regulation in this most important endocrine cell.

HIT-T15 cell line was used to examine the effect of protein kinase C on the cytosolic free $Ca^{2+}$ concentration and the activity of $Ca^{2+}$-activated $K^+$ channels. Results indicate that the activation of protein kinase C is involved in the glucose-induced release of insulin by modulating $K^+$ channel function in HIT-T15 cells (15). Hughes et al. studied the contribution of signal-transduction pathways to acetylcholine-induced insulin release in the clonal ß-cell line HIT-T1 5 (16).

These cells were used to study to provide biochemical evidence which substantiates the traditional claims for an oral hypoglycaemic effect of herbs. The insulinotropic action of *Tinospora crispa* was investigated *in vitro* using isolated human or rat islets of Langerhans and HIT-T15 cells (17).

The aqueous extract of *Tinospora crispa* sensitizes the clonal ß-cell line, HIT-T15 to extracellular $Ca^{2+}$ and promotes

intracellular $Ca^{2+}$ accumulation which in turn causes increased insulin release (18).

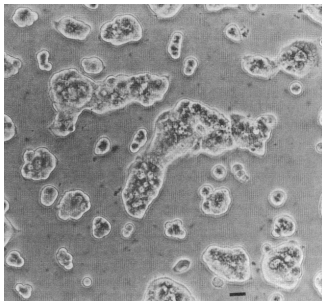

*Figure 3: Light micrograph of 4-day HIT-T15 culture. Phase contrast. (Bar = 20 µm.)*

*In vitro* procedure for identifying herbs potentially possessing sulfonylurea-like activity was reported by Rotshteyn et al. They showed the hypoglycemic properties of bitter melon (*Momordica charantia*, Linn. Family, Cucurbitacea), cerasse (*Momordica charantia*, Linn. wild variety, Family, Cucurbitacea) and American ginseng (*Panax quinquefolius*, Linn., Family Araliacea) were at least partially due to their sulfonylurea-like activity. The procedure consisted of the combination of an SUR1 (Sulphonylureareceptor 1) binding assay and an insulin secretion assay in cultures of HIT-T15 cells (19). The high molecular weight fraction and the low molecular weight fraction of *Mormordica Charantia Linn*. was investigated for their cell reparative effects and insulin secretagogue effect on the HIT-T15 Hamster Pancreatic ß-cells (20). The protective effect of *Amomi semen* extract (ASE) on alloxan-induced pancreatic *ß*-cell damage was investigated in HIT T-15 cells. The results of this study provide evidence that ASE may have a protective activity on alloxan-induced ß-cell damage, and that the protective effect is primarily due to the inhibition of Reactive Oxygen Species (ROS) generation by alloxan (21). Deeney et al. described the modulatory role of protein kinase C in insulin secretory process using HIT-T15 cell line (22).

The studies described that chronic exposure of HIT-T15 ß cells to supraphysiologic glucose concentrations leads to decreased expression of the STF-1 (homeoprotein closely related to the Xenopus XlHbox 8 protein) transcription factor by altering the posttranslational processing of STF-1 RNA. The lack of STF-1 may contribute to the decrease in insulin gene transcription observed in these cells and it leads to adverse glucotoxic effects on insulin gene expression in pancreatic ß cells (23). This cell line has also been used to study the muscarininc modulation of voltage-dependent $Ca^{2+}$ channels to derive the mechanism of insulin secretion from pancreatic ß-cells (24).

***BRIN BD-11***

A novel insulin-secreting cell line (BRIN-BD11) was established after electrofusion of RINm5F cells with New England Deaconess Hospital rat pancreatic islet cells. This hybrid insulin-secreting cell lines offer many merits, in a view that they are able to be grown up in very large numbers and, with long-term stability in tissue culture, offer potentially useful model *ß*-cells, it was theorized that fusing normal pancreatic *ß*-cells with a cultured cell line, such as RINm5F, would provide a source of novel *ß*-cell clones. As such, electrofusion-derived hybrid cells should obtain immortality from the RINm5F fusion partner and intact features of insulin biosynthesis and secretion from the normal parental pancreatic *ß*-cells. Adopting this novel approach, three clonal rodent cell lines were established following electrofusion of New England Deaconess hospital (NEDH) rat *ß*-cells with immortal RINm5F cells, originally derived from the transplantable NEDH rat insulinoma (25, 26 ). Procedures adopted for the isolation of these three clonal *ß*-cells (denoted BRIN-BD11, BRIN-BG5 and BRIN-BG7) were described by McClenaghan *et al*. The BRIN nomenclature comes from the B-cell-RIN cell fusion, with each cell line cloned from well B into three wells: D11, G5 and G7. Cultured BRIN cells form monolayers, with epithelial cell morphology, taking on a pavemental pattern when confluent. Wells of cell fusion mixture with insulin output 5-10 times greater than parent RINm5F cells were subcultured with eventual establishment of clones, including BRIN-BD11. Morphological studies established that these cells grow as monolayers with epithelioid characteristics. Notably, BRIN-BD11 cells are phenotypically and functionally stable for over 4 months (50 passages) in culture and, although they do not match the granulation, having less than 5% of the insulin content of primary rat *ß*-cells, importantly they demonstrate regulated insulin release. Western blotting confirmed that BRIN-BD11 cells expressed the glucose transporter (GLUT2). This, coupled with a high glucokinase/hexokinase ratio in the cells, confirms an intact glucose sensing mechanism. High-performance liquid chromatography analysis demonstrated that insulin was the major product secreted under stimulatory conditions. Collectively, these data indicate that the BRIN-BD11 cell line represents an important stable glucose-responsive insulin-secreting beta-cell line for future studies. Early cell lines, such as RINm5F and HIT-T15, were rather crude proxy for normal *ß*- cells, the advances in molecular biology and emergence of novel bioengineering technologies have provided new opportunities to improve and establish more appropriate cultured cell lines (27). With the highest GLUT2 protein expression and a high glucokinase:hexokinase ratio, the BRIN-BD11 cells showed the most impressive glucose-sensing ability (25), which translated into a stepwise increase in insulin release in response to 4.2– 16.7 mmol glucose. While most insulin-secreting cell lines are either unresponsive, as in the case of parental RINm5F cells, or respond to subphysiological glucose concentrations, the threshold for glucose-stimulated insulin release in BRIN-BD11 cells is as reported in normal *ß*-cells, and these cells demonstrate a strong first phase insulin release with sustained release at a level marginally higher than basal, corresponding to the second phase (25). The importance of glucokinase:hexokinase ratio to effective glucose-sensing is illustrated by the features of BRIN-BD11cells(25, 29) and through establishment of glucose-stimulated insulin release in RINm5F cells by overexpression of glucokinase (30).

D-glucose metabolism when was compared in BRIN-BD11 and RINm5F cells, extends the knowledge that BRIN-BD11 cells display an improved metabolic and secretory behavior, when

considering the difference otherwise found between normal and tumoral islet cells. BRIN-BD11 cells were less affected than RINm5F cells by a rise in D-glucose concentration, in terms of the inhibitory action of the hexose upon oxidative variables, such as oxidative glycolysis, pyruvate decarboxylation, and oxidation of glucose-derived acetyl residues in the Krebs cycle (31).

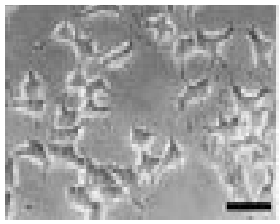

*Figure 4: Cellular morphology BRIN-BD11 cell*

BRIN-BD11 cells have been characterized in detail, and express many key features of normal pancreatic ß-cells (27, 28), including the two-component $K_{ATP}$ channel (Kir6.2 and SUR1), VDCCs, and elements regulating late stages of the insulin secretory pathway, including phospholipase C (PLC)/protein kinase C (PKC) and adenylate cyclase (AC)/protein kinase A (PKA). BRIN-BD11 cells have also been useful in studies probing so-called $K_{ATP}$ channel-independent actions of glucose, nutrients and insulinotropic drugs (28) and have also been used in investigations of ß-cell dysfunction, demise and destruction (32-34). With relatively lower granulation, BRIN-BD11 cells may be less useful for in-depth study of the processes underlying exocytosis, although it is unrealistic to expect a bioengineered clonal cell to exactly match a freshly isolated cell. In this regard, however, the many merits evident from the characterization and use of BRIN-BD11 cells in diverse functional studies outlined in this review should be balanced against the short lifespan of primary ß-cells, which also suffer from a rapid decline in function and granulation with time in culture.

This cell line responds to a wide variety of insulinotrophic stimuli including glucose, amino acids, hormones, neurotransmitters and drugs (25, 35-37). The appropriateness of BRIN-BD11 cells for screening of antidiabetic plant materials and characterization of novel insulin-releasing natural products has also been described (38-40). Acute and chronic effects of the insulinotropic drug nateglinide upon insulin release has been examined in the BRIN-BD11 cell line. The data showed that nateglinide stimulates both $K_{ATP}$ channel-dependent and-independent insulin secretion. The maintained insulinotropic effects of this drug with increasing glucose concentrations support the antihyperglycaemic actions of nateglinide in Type II diabetes. Studies of the long-term effects of nateglinide indicated that nateglinide shares signalling pathways with sulphonylureas, but not the imidazoline efaroxan. This may be significant when considering a nateglinide treatment regimen, particularly in patients previously treated with sulphonylurea (41).

BRIN-BD11 cells has been implanted intraperitoneally into severely hyperglycaemic (>24mmol/1) streptozotocin-induced insulin-treated diabetic athymic nude (nu/nu) mice. The implants reduced hyperglycaemia such that insulin injections were discontinued by 5-16 days (<17mmol/L) and normoglycaemia (<9mmol/L) was achieved by 7-20 days. Implanted cells were removed after 28 days and re-established in culture. After re-culture for 20 days, glucose-stimulated (16.7mmol/L) insulin release was enhanced by 121% compared to non-implanted cells (42).

McClenaghan studied glucose-sensing mechanism (43), permissive effect of and non-glucidic nutrients (44), actions of keto acid initiators of insulin secretion (45), hexose recognition (46), relationship between dietary carbohydrate intake and insulin resistance (47), and glucose-dependent insulin secretory response (50, 51) in BRIN BD 11 cell line.

***MIN 6***

MIN6 is mouse insulinoma cells. This cell line was established from insulinomas obtained by targeted expression of the simian virus 40 T antigen gene in transgenic mice. Two cell lines were established at the instance. These cell lines, designated MIN6 and MIN7, produce insulin and T antigen and were morphological characteristics of pancreatic beta cells. MIN6 cells exhibited glucose-inducible insulin secretion comparable with cultured normal mouse islet cells, whereas MIN7 cells do not. Both cell lines were producing liver-type glucose transporter (GT) mRNA at high level. Brain-type GT mRNA was also present at considerable level in MIN7 cells, but was barely detectable in MIN6 cells, suggested that exclusive expression of the liver-type GT is related to glucose- inducible insulin secretion (53). MIN6 exhibit characteristic of glucose metabolism and glucose stimulated insulin secretion similar to those of normal islets (54). MIN6 cells did not express either major histocompatibility (MHC) class I or class II antigens on the cell surface. Glucose induces both calcium-dependent and calcium-independent insulin secretion from the pancreatic beta cell line MIN6 (55).

MIN6 cell line has been studied well for establishing an importance of mitochondrial DNA and ATP production in insulin secretion induced by secretagogues like glucose and leucine. Mitochondrial DNA-depleted pancreatic-cell line found to have impaired insulin secretion induced by glucose, leucine and sulfonylureas (56). On insulin secretion in response to glucose and other nutrients, mitochondrial oxidative phosphorylation and the production of ATP in pancreatic beta cells have been proposed to play significant roles (57). ATP production and subsequent increase in the ATP/ADP ratio block ATP sensitive $K^+$ channels, resulting in depolarization of the membrane potentials of the pancreatic beta cells, followed by the influx of extracellular $Ca^{2+}$ into the cells. As a consequence, the intracellular free $Ca^{2+}$ concentration increases, and the cells secrete insulin through exocytosis. Therefore, the impairment of oxidative phosphorylation in mitochondria could be the cause of diabetes mellitus through defective insulin secretion (58).

Inada et al., reported the successful transplantation of MIN6 cell in C57BL/KsJ mice. A pancreatic beta cell line MIN6 was transplanted subcutaneously into the back of the mice. A study of 100 days suggested restoration of blood insulin level, reduction of blood glucose level, absence of glucose tolerance

and fatty liver implicated its potential in diabetes therapy (59). MIN6 cell line has been used as a bioartificial endocrine pancreas for xenoimplantation (60, 61). MIN6 cell line has also been used to screen many hypoglycemic agents that works through insulin secretogogue action (62-64).

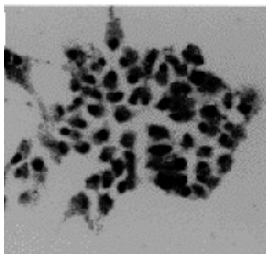

*Figure 5: Histochemical staining of mitochondrial enzymes in MIN6 cells, photographed through a light microscope (X 200).*

***INS-1***

Cloned cell line like RINm5F- 2A has retained many characteristics of normal ß-cells, but they lost certain prominent features of the parent cell, including the capacity to secrete insulin in response to glucose and to synthesize and store normal levels of insulin (65). The HIT cell, another commonly used ß- cell line established by transformation of hamster islet cells by SV40, displayed glucose-induced insulin release well as glucose responsiveness with increasing passage numbers (66, 67).Many, other cell lines have also been generated from pancreatic islets tumors of transgenic mice (68-70). Although the ß -TC lines from transgenic mice maintain many aspects of ß-cell function, including glucose sensitivity, they too lose their differentiated states as a function of tissue culture passage (68). Experimentation with both HIT and ß -TC cells, therefore, has constraints in regard to cell stability and required close monitoring of their functions. Accordingly, the search for new stable ß-cell lines still remains an important task. Furthermore, it has proved difficult to establish human ß-cell lines from insulinoma (or nesidioblastosis) tissue. The availability of such cells could be of obvious importance for diabetes research.

Insulin secreting cell line INS-1 was established from cells isolated from an x-ray-induced rat transplantable insulinoma. Wollheim CB et al., observed the formation of free-floating and slowly growing cell aggregates in the course of co-culture experiments between lymphocytes and cells freshly dispersed from the x-ray induced rat insulinoma (71). It was maintained by serial SC transplantation into New England Deaconess Hospital (NEDH) rats (72). The continuous growth of these cells is dependent on the reducing agent 2- mercaptoethanol. Removal of this thiol compound causes a 15- fold drop in total cellular glutathione levels. These cells proliferates slowly (population doubling time about 100 h) and, in general, shows morphological characteristics typical of native ß-cells. INS-l cells show an ultrastructural organization typical of epithelial and peptide-producing cells. The characteristic electron microscopic appearance of some of their secretory granules and their positive immunofluorescence labeling by specific antibodies indicate that INS-l cells store insulin. Most cells stains positive for insulin and they do not react with antibodies against the other islet hormones. The content of immunoreactive insulin is about 8 µg/$10^6$ cells, corresponding to 20% of the native ß-cell content. This cell synthesizes both proinsulin I and II and display conversion rates of the two precursor hormones similar to those observed in rat islets (73).

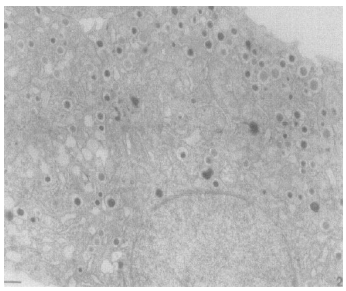

*Figure 6: Ultrastructural organization of INS-1 cells from a 1-week-old culture. The appearance of the INS-1 cells illustrated in this field is virtually indistinguishable from that normally displayed by the insulin-producing β-cells of the islets of Langerhans.*

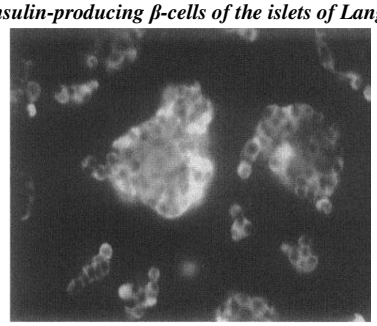

*Figure 7: Immunofluorescence staining of INS-l cultures for insulin. The nuclei of INS-l cells were not labeled by fluorescence.*

INS1 cell line has become a useful model for studying the molecular mechanism of $Ca^{2+}$- dependent and $Ca^{2+}$-independent insulin secretion (74). INS-1 cells have retained the capability to respond to an increase in the glucose concentration with a biphasic insulin secretion (75). Most important is the observation that INS-1 cells secrete insulin in response to glucose concentrations in the physiological range. In addition, INS-1 cells can be genetically engineered, which is useful for basic research as well as for exploring their use as an alternative to isolated islets for transplantation therapy of type 1 diabetes (76).

***Skeletal muscle cell line:***

***L6 myogenic cell line***

L6 cells, originally derived from rat skeletal muscle, cultured from the thigh of a one day old rat, propagate as mononucleated myoblasts but differentiate by spontaneous cellular fusion into multinucleated primary myotubes (77). Insulin stimulation of glucose transport in L6 muscle cells results predominantly from the translocation to the cell surface of the glucose transporter GLUT4 (78, 79). The myotubes express several proteins typical of skeletal muscle including the GLUT4 glucose transporter (79-82). Insulin stimulates glucose uptake with high sensitivity and maximal responsiveness only in differentiated L6 myotubes; GLUT4

expression parallels the acquisition of these characteristics as the L6 cells differentiate (77, 78-82). These features of L6 myotubes are important since GLUT4 is responsible for insulin-dependent glucose uptake in mature skeletal muscle. In the myotube stage, GLUT4 coexists along with the housekeeping glucose transporter GLUT1 and the fetal muscle transporter GLUT3. These cells have a fully functional insulin-signalling cascade including robust activation of Akt.

The inventors have subcloned an L6 myoblast cell line that was selected for high fusion capacity as they differentiate into myotubes (77). Glucose uptake in L6 myotubes is stimulated by insulin with a 1.5 to 2-fold increase of their maximal response above basal (unstimulated) rates (77, 78). Glucose uptake in L6 cells also responds rapidly to stimulation by IGF-I in much the same manner as insulin (a 2-fold maximal stimulation above basal) (83). In addition, prolonged exposure of L6 cultures to insulin or IGF-I induce hypertrophy of L6 myotubes, glucose transporter biosynthesis, and 3-4 fold increases in glucose uptake (83, 84). Insulin-stimulated glucose uptake measurements in isolated rat and mouse EDL and soleus muscle are typically 3 to 4-fold and 2 to 3-fold above basal rates, respectively. Thus, L6 myotubes in culture have a significant glucose uptake response to insulin that is within the range established for isolated rodent skeletal muscle preparation (85).

Hypoxic or anaerobic conditions, *in vivo*, are typically encountered by exercising muscle and in many ways mimic the stimulation of glucose uptake caused by muscle contraction during exercise (86). Exercise-stimulated glucose uptake is mediated by GLUT4 and utilizes a different signal transduction pathway and possibly a separate intracellular pool of GLUT4 glucose transporters compared to insulin-responsive glucose transport (87). L6 myotubes have hypoxia-responsive glucose uptake and in this manner also provide a surrogate model for exercise-stimulated glucose uptake (88, 89). An antiproteolytic effect of glyburide has been studied by measuring the release of $^{14}$C-tyrosine from intact L6 myoblasts (90).

The technology provides a L6 muscle cell line that can differentiate with high reliability into a myotube muscle cell phenotype that naturally expresses the GLUT4 glucose transporter protein, and has a significant insulin-stimulated glucose uptake biological response. The cell line can be used as the basis for a high throughput screen in the search for novel anti-diabetic compounds by measuring their effect on glucose uptake. The cell line thus provides an efficacious alternative to isolated skeletal muscle tissues or primary skeletal muscle cell cultures.

The L6 myotube cell line is the best-characterized cellular model of skeletal muscle origin to study glucose uptake and GLUT4 translocation. The cell line is amenable for transient transfection by plasmid-based gene transfer (91, 92) and viral infection protocols (retrovirus and adenovirus). Stable transfectants can be differentiated into myotubes. Adenovirus infection can be performed in myotubes. Augmentation of the effects of insulin and insulin like growth factor I and II on glucose uptake by sulfonylureas (93), coordinate regulation of glucose transporter function, and gene expression by insulin and sulfonylureas (94), glyburide-stimulated glucose transport via protein kinase C-mediated pathway (95) were studied in this cellline.

***BC3H1***

A primary cultures for smooth muscle cells is difficult to maintain for a longer period of time. $BC_3H1$ cell line was first developed by Schubert et al.(96) which has been derived from a neoplasm induced with nitrosoethyl urea (NEU) in the $C_3H$ mouse strain. Since then it has been suggested that BC3H1 is a skeletal line of ectodermal origin that is defective for commitment to terminal differentiation. The two distinct morphologies typical of this cell line are large spheroid cells and smooth-muscle-like cells. The fusiform is the more common. This myogenic cells shares many properties with smooth muscle. The cells have electrically excitable membranes capable of generating overshooting action potentials, and they contract both spontaneously and with electrical stimulation. They respond to the iontophoretic application of acetylcholine with a depolarizing response, and to norepinephrine with a hyperpolarizing response. Electron microscopy reveals that the cells have morphology similar in many, but not all, respect to that of smooth muscle cell *in vivo*. The cells secrete soluble collagen like molecule in addition to several proteins of undefined function. The $BC_3H1$ cell line has been used widely as a model for studying regulation of muscle-related proteins, such as the acetylcholine receptor, myokinase, creatine kinase, and actin (97).

BC3H1 cells accumulate a variety of muscle specific gene products, such as muscle creatine phosphokinase (MCK) (96; 98; 99), myokinase (96), and a-actin (100, 101) as well as the nicotinic acetylcholine (102) and insulin receptors (103).

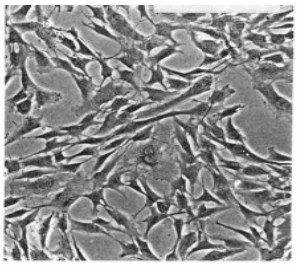

***Figure 8: Morphology of BC3Hl cells***

These cells spontaneously differentiate postconfluence from myoblastst to nonfusing myocytes, as demonstrated by cell elongation and increased muscle-specific proteins this differentiation process is associated with the development of high affinity insulin receptors, accompanied by insulin-stimulated responses and receptor regulation which are both sensitive to physiological hormone concentrations. Furthermore, this cell system avoids several of the difficulties of other systems, including the artifactual receptor regulation (104, 105) and unpredictability of collagen digested adipocytes, the pharmacological requirements (106) and questionable receptor down-regulation of differentiated 3T3-Ll fibroblast/adipocytes (107-111) and the high degradative activity and absence of insulin-stimulated glucose transport in hepatocytes. Therefore, this continuously cultured muscle cell system presents a unique model for insulin action in

muscle and for the development of insulin receptors and responses as a function of target tissue differentiation.

In the study carried out by Mayor et al., they examined regulation of glucose transport in $BC_3H1$ myocytes as a model for muscle. In myocytes, chronic glucose exposure per se (25 mmol) decreased basal glucose transport activity by 78% and insulin's acute ability to maximally stimulate transport by 68%. Chronic glucose exposure also reduced cell surface insulin binding by 30% via an apparent decrease in receptor affinity, and this effect was associated with a comparable rightward shift in the insulin-glucose transport dose-response curve. In other studies, persistent stimulation with 15 nmol insulin also decreased maximally stimulated glucose transport activity, which was independent and additive to the regulatory effect of glucose. Moreover, glucose and insulin, induced insulin resistance via different mechanisms. Glucose (25 mmol) reduced the number of cellular glucose transporter proteins by 84% and levels of GLUT1 transporter mRNA by 50%. In contrast, chronic insulin exposure led to a 2.1-fold increase in GLUT1 mRNA but did not alter cellular levels of transporter protein. Cotreatment with glucose prevented the insulin-induced rise in GLUT1 mRNA. $BC_3H1$ cells did not express GLUT4 mRNA that encodes the major transporter isoform in skeletal muscle. In conclusion, in $BC_3H1$ myocytes glucose diminished insulin sensitivity by decreasing insulin receptor binding affinity and decreased basal and maximally insulin-stimulated glucose transport rates via cellular depletion of glucose transporters and suppression of GLUT1 mRNA. Chronic insulin exposure to $BC_3H1$ cell line exerted an independent and additive effect to reduce maximal transport activity, hence although $BC_3H1$ cells are commonly used as a model for skeletal muscle, studies examining glucose transport should be interpreted cautiously due to the absence of GLUT4 expression (112).

Direct effects of sulfonylurea agents on glucose transport (113), insulin-indused decrease in 5'-nucleotidase activities in skeletal muscle membrane (114), sulfonylurea-stimulated glucose transport association with deacylglycerol-like activation of protein kinase C (115) were studied in this cell line. *In vitro* studies on antidiabetic agent has also been carried out using $BC_3H1$ muscle cell (116).

***C2C12 Cell line***

C2C12 cells were originally obtained by Yaffe and Saxel (117) through selective serial passage of myoblasts cultured from the thigh muscle of C3H mice 70 h after a crush injury. These cells were shown to be capable of differentiation. C2C12 cells are a useful model to study the differentiation of non-muscle cells to skeletal muscle cells (e.g myosin phosphorylation mechanisms) and express muscle proteins and the androhen receptor (AR).

Biophysical, biochemical, and immunocytochemical properties of C2C12 cells were measured by Mc Mohan et al (118). It demonstrate that cardiac and skeletal muscle troponin T isoforms are incorporated and colocalized into myofibrils which suggest that these cells could be a useful model to assess the effects of exogenous native and mutated cardiac and skeletal contractile protein isoforms on myofilament function. A mouse skeletal muscle cell line, have been shown to be suitable for stable transfection experiments of exogenous cDNA, making this cell line a candidate for stable transfection of cDNAs that encode mutant skeletal muscle and cardiac protein isoforms. This cell culture found an instrumental role in the study of human insulin resistance and in investigations on the mechanism of action of antidiabetic drugs. Insulin stimulated 2-deoxyglucose uptake was well studied in this cell line (119, 120).

Cells of the C2C12 mouse myoblast cell line were also used to examine the effects of various agents on the hormonal regulation of IGF-binding protein-2 (IGFBP-2) secretion and mRNA expression in myoblasts. The findings suggested that multiple factors, including growth factors and metabolic hormones were involved in regulating IGFBP-2 in C2C12 myoblasts (121).

Ming Li et al. studied the effect of Chinese herb Geum japonicum, on the processes of angiogenesis and cardiomyogenesis. They investigated these dual properties in *ex vivo* by testing the different fractions of herb to check their ability to stimulate proliferation of C2C12 myoblast (122). The mechanism of the plasma glucose lowering action of andrographolide was investigated using radioactive glucose uptake into cultured myoblast C2C12 cells as the indicator. In C2C12 cells, andrographolide found to increase the radioactive glucose uptake in a concentration-dependent manner that was abolished by pretreatment with prazosin. Activation of alpha1-adrenoceptors by andrographolide was further indicated by the displacement of the [3H] prazosin binding in C2C12 cells (123). Same kind of study has been undertaken for soyabean isoflavone genistein to inhibit tyrosine kinase in cultured C2C12 cells (124). The effects of insulin-like growth factor-I (IGF-I) on cellular responses of primary human skeletal muscle cells and mouse C2C12 myoblasts have been investigated (125). Insulin sensitizing action of metformin on skeletal muscle cells has been evaluated by using C2C12 skeletal muscle cells. The study demonstrated the direct insulin sensitizing action of metformin on skeletal muscle cells (126). A study undertaken to evaluate the role of gliclazide, a second generation sulphonylurea, to enhance insulin signaling in insulin-resistant skeletal muscle cells (C2C12 cells). The study proposed that gliclazide can regulate part of the insulin signaling in insulin-resistant skeletal muscle by enhancing insulin-stimulated insulin receptor tyrosine phosphorylation in insulin-resistant skeletal muscle cells (127).

Peroxisome proliferator-activated receptor γ (PPAR- γ)) is a member of the nuclear receptor superfamily known to regulate adipocyte differentiation. Its role in skeletal muscle differentiation was investigated to know the possible involvement of PPAR- γ in skeletal muscle differentiation. Modulated PPAR- γ expression in C2C12 mouse skeletal muscle cells by stable transfection with sense or antisense plasmid constructs of PPAR- γ cDNA found to inhibit myogenic differentiation in C2C12 skeletal muscle cells (128). PPAR- γ expression is very low in skeletal muscle cells, which is one of the most important target tissues for insulin and plays a predominant role in glucose homeostasis. It has recently been shown that muscle-specific PPAR- γ deletion in mouse causes

insulin resistance (129, 130). Role of muscle PPAR-γ in insulin sensitivity has explored by Verma NK et al. The study demonstrated a direct involvement of PPAR- γ in insulin sensitization of thiazolidinedione action on skeletal muscle cells, and suggested that pharmacological overexpression of muscle PPAR-γ gene in skeletal muscle might be a useful strategy for the treatment of insulin resistance (131).

***Adipocytes:***

***3T3 L1***

Insulin resistance and obesity are hallmarks of NIDDM. As one of the targets of insulin action, adipose tissue plays an important role in maintaining whole body energy homeostasis. The 3T3-L1 cells are routinely used in the signaling studies and are regarded as demonstrating all of the features of adipocytes.

3T3 cell line established in 1962 by two scientists George Todaro and Howard Green at the department of pathology in the New York University school of medicine. The 3T3 cell line has become the standard fibroblast cell line thereafetr (132). The name '3T3' refers to the abbreviation of "3-day transfer, inoculum 3 x $10^5$ cells." This cell line was originally established from the primary mouse embryonic fibroblast cells. The primary mouse embryonic fibroblast cells were transferred (the "T") every 3 days (the first "3"), and inoculated at the rigid density of 3 x $10^5$ cells per 20-$cm^2$ dish (the second "3") continuously. The spontaneously immortalized cells with stable growth rate were established after 20-30 generations in culture, and then named '3T3' cells.

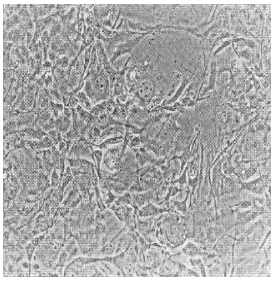

***Figure 9: 3T3-L1 Preadipocytes***

3T3 cells are often used in the cultivation of keratinocytes, with the 3T3 cells secreting growth factors favourable to these kinds of cells. Glucose entry into cells is shown to be facilitated by glucose transporter proteins belonging to a family of five homologous members, each with specific tissue distributions (133, 134). One member of this family, GLUT4, is an integral membrane protein expressed only in tissues in which glucose uptake is regulated by insulin i.e. fat, skeletal muscle, and heart. Adipocytes normally play an important role as a major site for systemic energy homeostasis, adipocyte function is markedly altered in disorders such as diabetes (135, 136). The subcellular distributions of the transporter isoforms GLUT1 and GLUT4 on cell surface of 3T3-L1 cells were determined by Yang et al (137).

Szalkowski et al. studied the effect of pioglitazone, ciglitazone and englitazone, (members of insulin-sensitizing thiazolidinedione derivatives) on 3T3 cells (138). Sandouk et al., investigated the effect of pioglitazone, on expression of glucose transporters GLUT1 and GLUT4 in 3T3-F442A cells (139). The effect of pioglitazone on cellular differentiation and expression of adipose-specific genes, adipsin and aP2 has been studied in 3T3-F44ZA cells. Findings indicated pioglitazone as a potent adipogenic (139). They showed that pioglitazone increases expression of glucose transporters in 3T3-F442A cells by increasing messenger ribonucleic acid transcript stability (140).

3T3-Ll preadipocytes were found to express mRNAs encoding type I, III, and IV procollagens. When 3T3-Ll cells were stimulated to differentiate into adipocytes, the relative concentrations of type I and type III procollagen mRNAs declined by 80-90%. Parallel decreases in the rates of transcription of the procollagen I and procollagen III genes appear to account for the diminished levels of these mRNAs. In contrast, the relative rate of transcription of the procollagen IV gene increased 2.6-fold during adipocyte development. As a consequence, the abundance of type IV procollagen transcripts was elevated in adipocytes. Tumor necrosis factor a (TNF- α) is a cytokine that stimulates lipolysis, an apparent “dedifferentiation” of adipocytes, and inhibits transcription of certain adipocyte-specific genes (141).Tumor necrosis factor-α (TNF- α) is recognized as playing a role in the development of insulin resistance in a variety of catabolic states, including septic shock, acute infection, and long-term tumor bearing. Infusion of rats with TNF-α resulted in marked insulin resistance, as measured by hepatic glucose output and peripheral glucose disposal. Stephens and Pekala studied the effect of tumor necrosis factor-α on Fully differentiated 3T3-L1 adipocytes. They conclude that tumor necrosis factor-alpha (5 nmol) resulted in the development of an insulin resistance based on the inability of insulin to stimulate hexose uptake with total depletion of GLUT4 protein (insulin-responsive glucose transporter) in isolated membrane of cells chronically treated with TNF-α (142). Ability of tumor necrosis factor-alpha to stimulate hexose transport has been examined in quiescent 3T3-L1 fibroblasts (143). Tumor necrosis factor treated 3T3-L1 adipocytes were used as a model for studying the effects of systemic inflammation on adipose tissue (144). Thiazolidinediones were studied to check their inhibitory effect of TNF-α on 3T3-Ll cell differentiation. Furthermore, the reduction in glucose uptake in response to insulin as well as selective down-regulation of GLUT4 and other specific adipocyte genes caused by long term incubation of 3T3-Ll adipocytes with TNF-α were ameliorated by thiazolidinedione treatment. Mechanism of action of thiazolidinedione group of compounds was evaluated in 3T3-L1 cell line.

Transcriptional regulation of Lipoprotein lipase gene (LPL is synthesized primarily in muscle and adipose tissue and by hydrolyzing triglycerides in chylomicrons and very low density lipoprotein allows uptake of the resultant free fatty acids by these tissues) was studied by Zachner et al. in mouse 3T3-L1 adipocytes. They found that recombinant human cachectin tumor necrosis factor down regulates lipoprotein lipase gene expression (145). Fryer et al., reported the effect of tumour necrosis factor on regulation of lipoprotein lipase mRNA content in 3T3-L1 cells. TNF reversibly down-regulates LPL mRNA in fully differentiated 3T3-L1 adipocytes (146). The

studies suggest that the cytokine could induce insulin resistance in adipocytes by decreasing the expression of GLUT4 and, subsequently, insulin-stimulated glucose uptake in NIDDM. It has been demonstrated that depletion of GLUT4 correlated positively with insulin resistance in patients with NIDDM.

Calderhead et al. reported the amounts of the brain type and muscle type glucose transporters (GLUT 1 and 4, respectively) in 3T3-L1 adipocytes by quantitative immunoblotting with antibodies against their carboxyl-terminal peptides. There are about 950,000 and 280,000 copies of GLUT 1 and 4, respectively, per cell. They evaluated the effect of Insulin on the translocation of both types of transporters from an intracellular location to the plasma membrane. They observed that insulin-elicited increase in cell surface transporters. The increases in GLUT 1 and 4 was averaged 6.5- and 17-fold, respectively (147). The stimulating effect of Insulin on hexose transport and phosphorylation of the insulin receptor was studied monolayer cultures of intact 3T3-L1 adipocytes (148). Clancy and Czech reported the stimulation of hexose transport and membrane redistribution of glucose transporter isoforms (GLUT1 and GLUT4) in response to cholera toxin, dibutyryl cyclic AMP, and insulin in 3T3-Ll adipocytes (149). Run-on transcription assays indicated a coordinate transcriptional repression of both GLUT4 and C/EBP-alpha genes(C/EBP-alpha has been suggested to control GLUT4 expression) as well as the 422/aP2 gene (the adipocyte lipid-binding protein, whose expression has also been proposed to be controlled by C/EBP-alpha (150).

Hydroxychalcone from cinnamon has been studied for its insulin mimetic action in 3T3-L1 adipocytes with regard to glucose uptake, glycogen synthesis, phosphatidylinositol-3-kinase dependency, glycogen synthase activation and glycogen synthase kinase-3ß activity (151). The ellagitannin, lagerstroemin was identified as an effective component of the banaba (*Lagerstroemia speciosa* L.) extract responsible for the activity. In a different approach, using 3T3-L1 adipocytes as a cell model and a glucose uptake assay as the functional screening method, Chen et al. showed that the banaba water extract exhibited an insulin-like glucose transport inducing activity (152). Glucose transport stimulatory and adipocyte differentiation inhibitory effect of tannic acid has been reported in 3T3-L1 Cells (153).

***Smooth muscle cell line:***

***Caco-2***

Caco-2 cell line is an epithelial cell line originates from human colon adenocarcinoma. The cell line forms a monolayer and easy to maintain. Caco-2 cells have been widely used in studies to determine the transport kinetics and metabolism of dietary polyphenols. Oral bioavailability of natural products like quercetin, quercetin 4′-glucoside, and quercetin 3,4′-diglucoside (154-156), chrysin and its conjugated metabolites (157), tea flavonoids(158), genistein (a soyabean derived isoflavone), daidzein and their glucosides (159, 160) has been studied using the Caco-2 human colonic cell line, a model of human intestinal absorption. Dietary D-Glucose is absorbed from the lumen of the small intestine by accumulation of D-glucose into enterocytes. This process is energized by simultaneous “downhill” transport of sodium ions and is mediated by the sodium-D-glucose cotransporter SGLT1. Therefore, the plasma membrane transporter SGLT1 is frequently expected to be located exclusively in the luminal brush-border membrane of enterocytes. A considerable amount of the sodium-D-glucose cotransporter SGLT1 present in Caco-2 cells, a model for human enterocytes, is located in intracellular compartments attached to microtubules (161). A similar distribution pattern was also observed in enterocytes in thin sections from human jejunum, highlighting the validity of the Caco-2 cell model. Fluorescent surface labeling of live Caco-2 cells revealed that the intracellular compartments containing SGLT1 were accessible by endocytosis. Studies under taken to compared SGLT1-mediated D-glucose uptake into Caco-2 cells with the subcellular distribution of SGLT1 after challenging the cells with different stimuli. Interestingly, regulation of SGLT1- mediated D-glucose uptake into Caco-2 cells by extracellular D-glucose levels occurred without redistribution of cellular SGLT1. A study using isoform-specific antibodies for glucose transporters (GLUT 1 through GLUT 5) Caco-2 cells expresses high level of GLUT 1, GLUT 3 and GLUT 5 (162).

The effect of different classes of dietary polyphenols on intestinal glucose uptake has been investigated using polarized Caco-2 intestinal cells where flavonoid glycosides and non-glycosylated polyphenols found to inhibit glucose uptake into cells under sodium dependent conditions and under sodium-free conditions, aglycones and non-glycosylated polyphenols inhibited glucose uptake (163).

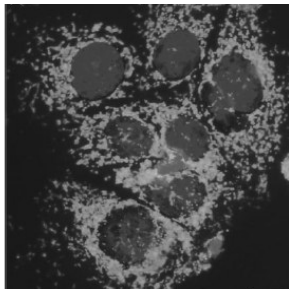

***Figure 10: Distribution of SGLT1 in Caco-2 cells: immunofluorescence microscopy. (DAPI staining).***

***Hepatic cell lines***

One of the hallmarks of diabetes is the inability of insulin to inhibit hepatic glucose production. It has been suggested that increased gluconeogenesis is a main source of increased hepatic glucose production and that the ability of insulin to regulate transcription of the rate-controlling gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), may contribute to this problem. This point is underscored by the observation that in several animal models of type II diabetes and obesity, PEPCK mRNA levels are increased 2–3-fold over that observed in non-diabetic animals, despite the higher circulating insulin levels observed in the diabetic animals (164-166). Also, transgenic mice that overexpress PEPCK display a diabetes-like syndrome (167). The rate of transcription of the hepatic PEPCK gene is increased by several hormones, including glucocorticoids, retinoic acid, and glucagon via its second messenger, cAMP

(168-171). Insulin dominantly represses PEPCK gene transcription (172-174) and the use of specific kinase inhibitors revealed that PI3K (phosphoinositide 3-kinase), but neither MAPK (mitogen-activated protein kinase) nor $p70^{S6K}$, is involved in the insulin response of the PEPCK gene (175). A variety of other agents is insulinomimetic in the sense that these compounds reduce PEPCK mRNA levels. Such compounds include phorbol esters, compounds that elicit oxidative and cellular stress (such as $H_2O_2$ and sodium arsenite), and the cytokines tumor necrosis factor-α interleukin-6, and interleukin-1. These agents differ from insulin, however, in that they repress PEPCK gene transcription in a PI3K-independent manner (176-179).

***Hep G2***

Hep G2 (Hepatocellular carcinoma, human) is a perpetual add cell line which was derived from the liver tissue of a 15 year old caucasian male with a well differentiated hepatocellular carcinoma. This cell line was first established by Knowles et al in 1980. Along with Hep G2, Hep 3B was also developed. These cell lines provide experimental models for investigation of plasma protein biosynthesis and the relation of the hepatitis B virus genome to tumorigenicity (180). The human hepatoma cell lines Hep G2 provide a unique model for studies of the regulation of human apoprotein and lipoprotein synthesis and catabolism (181). These cells are epithelial in morphology, have a model chromosome number of 55 and are not tumorigenic in nude mice. The cells secrete a variety of major plasma proteins e.g. albumin, alpha 2-macroglobulin, alpha 1-antitrypsin, transferrin and plasminogen. They have been grown successfully in large scale cultivation systems. Hepatitis B virus surface antigens have not been detected. The cells will respond to stimulation with human growth hormone.

When Cultures of primary hepatocytes and hepatoma cell line Hep G2 were compared human hepatocytes were found to be preferred model for biotransformation in human liver, whereas HepG2 cells to study regulation of drug-metabolizing enzymes (182).

Since the Hep G2 cells are immortal and resistant to cryopreservation, their usage offers advantages compared to primary liver cells in terms of availability, growth activity and quality control (183). Studies have established on Hep G2 cells that ethanol can induce apoptosis in liver cells (184-186). This cell line has been exploited to study the mechanism underlying ethanol-induced apoptosis in liver cells. Result indicated that the metabolism of sphigmomyelin played an important role in ethanol-induced apoptosis in Hep G2 cells. Activation of neutral sphigmomyelinase participates in ethanol-induced apoptosis in Hep G2 cells (187-189). The effect of Epigallo catechin gallate on tyrosine phosphorylation of the IGF-1 receptor (IGF-1R) was also examined on Hep G2 cells (190). An orally active antidiabetic agent, was studied *in vitro* to evaluate its effect on carbohydrate metabolism in liver cells (116).

Hep G2 cell line has also been utilized to assess the metabolic and toxicological characteristics of CYP2E1 (Human cytochrome P-450 2E1, which takes part in the biotransformation of ethanol, acetone, many smallmolecule substrates and volatile anesthetics and also involved in chemical activation of many carcinogens, procarcinogens, and toxicants). Researcher cloned CYP2E1 cDNA and established a HepG2 cell line stably expressing recombinant CYP 2E1 (191).

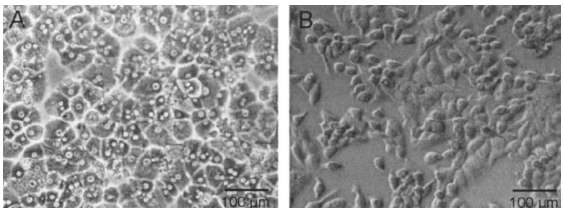


***Figure 11: Morphological comparison of human hepatocytes and hepatoma cell line HepG2. Phase-contrast micrographs (100X magnitude) show primary hepatocytes in culture (A) and HepG2 (B). Primary hepatocytes display the typical cubic cell shape, and HepG2 cells show rather an epithelial-like morphology.***

***H4IIE***

The continuous cell line, H4IIE, was derived from the Reuber Hepatoma H-35 (Reuber 1961) by Pitot and coworkers (192).

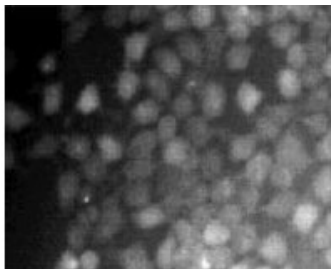

***Figure 12: Morphology of H4IIE cells.***

The regulation of hepatic glucose production is found to be decreased by green tea flavonoid, epigallocatechin gallate. This study was under taken on H4 IIE rat hepatoma cells. Further investigation revealed that epigallocatechin gallate act by increasing tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1 (IRS-1), and it as reducing phosphoenolpyruvate carboxykinase gene expression in a phosphoinositide 3-kinase-dependent manner. It also mimics insulin by increasing phosphoinositide 3-kinase, mitogen- activated protein kinase, and $p70^{S6K}$ activity. It also found to regulate genes that encode gluconeogenic enzymes and protein-tyrosine phosphorylation by modulating the redox state of the cell (190). H4 IIE cell line here provides a base to evolve the mechanism of action of the hypoglycemic agents.

**CONCLUSION**

Many animal models are available with features of type 2 diabetes and have allowed experimentation that would be impossible in humans. Even then, none of the known single species is exactly equivalent to human diabetes, but each model act as essential tool for investigating genetic, endocrine, metabolic, morphologic changes and underlying etiopathogenic mechanisms that could also operate during the evolution of type 2 diabetes in humans. Hence, care must be taken in interpretation and extrapolation of the results obtained from the animal models to humans. Further, the selection of particular animal model depends on the investigator's choice whether to use inbred or outbred,

availability of particular strain, aim of scientific strategy, type of drug being sought, and institutional financial and facility resources in the type 2 diabetes research. This is also dependent on the type of the programme of pharmaceutical drug discovery and development. In the screening of anti-diabetic compounds using cell lines, it is particularly important to note that some cell lines are better suited to screen particular class of anti-diabetic compounds based on their mechanism of action. Since drug discovery from both natural and also from the synthetic sources generally require screening and testing of large numbers of compounds in the industrial research environment, use of cell lines prove economical in producing test materials during some advanced studies like toxicity determination which requires invasive procedures and large blood and tissue samples in case of animals. The Screening studies using *in vitro* cell line models may also be less time consuming and more accurate and also provide better understanding of the mechanism/s of action in much closely similar human situation as well as for discovering new targets and drugs for the treatment of type 2 diabetes with a clear depiction of any complications whatsoever may arise.

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