# THE EFFECT OF HIGH FAT DIET &HIGH FRUCTOSE INTAKE ON INSULIN RESISTANCE AND GLP-1 IN EXPERIMENTAL ANIMALS

THESIS
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# LIST OF ABREVIATION

| | |
|---|---|
| **AC** | Adenyle cycles. |
| ACC | Acetyl-CoA carboxylase**.** |
| **ADA** | American Diabetes Association **.** |
| **AGEs** | Advanced glycosylated end products. |
| **ANOVA** | Analysis of variance |
| **ATP** | Adenosine tri phosphate **.** |
| **BMI** | Body mass index**.** |
| **cAMP** | cyclic adenosine mono phosphate. |
| **cAMP-GEF** II | cAMP binding guanine nucleotide exchange factor II. |
| **CAT** | Chloramphenicol-acetyltransferase**.** |
| **CD 220** | Cluster of differentiation. |
| **CHO** | Carbohydrate**.** |
| **CNS** | Central nervous system. |
| **CVD** | Cardiovascular disease**.** |
| **DPP-IV** | Dipeptidyl-peptidase-IV. |
| **DM** | Diabetes mellitus. |
| **ELISA** | Enzyme linked immuno sorbant assay |
| **FAS** | Fatty acid synthase **.** |
| **FFAs** | Free fatty acids**.** |
| **$GAD_{65}$** | Glutamic acid decarboxylase. |
| **GDM** | Gestational diabetes mellitus. |
| **GHRH** | Growth hormone releasing hormone. |
| **GPCR** | Guanine nucleotide-binding protein (G-protein) coupled receptor. |
| **GIP** | Glucose insulino tropic peptide. |
| **GLP-1** | Glucagon-like peptide-1. |
| **GLP-1R** | GLP1 receptor. |
| **GLUT4** | Glucose tansporter 4. |
| **Grb2/Sos** | Son of sevenless GDP exchange factor**.** |

| | |
|---|---|
| **HbA** | Haemoglobin A . |
| **HC** | High-carbohydrate diet**.** |
| **HDL** | High-density lipoprotein. |
| **HDL-C** | High-density lipoprotein-cholesterol**.** |
| **HFD** | High-fat diet**.** |
| **HFS** | High fat sucrose**.** |
| **HNFs** | Hepatocyte nuclear factors. |
| **HPA** | Hypothalamus-pituitary-adrenal axis. |
| **HR** | Horseradish peroxidase**.** |
| **HSL** | Hormone sensitive lipase **.** |
| **K+ATP** | ATP sensitive K+ channel**.** |
| **Kir-GEM** | Kinase –inducable rac –like protein GTP binding over expressed in skeletal muscles. |
| **IFG** | Impaired fasting glucose**.** |
| **IGT** | Impaired glucose tolerance. |
| **IMTG** | Intra muscular triglycerides**.** |
| **IPF-1** | Insulin promoter factor-1**.** |
| **IRS** | Insulin receptor substrate**.** |
| **INSR** | Insulin receptor**.** |
| **LDL** | Low-density lipoprotein. |
| **MAP kinase** | Mitogen-activated protein kinase. |
| **MI** | Myocardial infarction. |
| **MIDD** | Maternally inherited diabetes and deafness. |
| **MKK** | MAPK kinase. |
| **MODY** | Maturity-onset diabetes of the young **.** |
| **MRDM** | Malnutrition related diabetes mellitus. |
| **mRNA** | Mesenger ribonucleic acid **.** |
| **NAD** | Nicotinamide adenine dinucleotide |
| **NEFAs** | Nonesterified free fatty acids. |
| **NEUROD1** | Neurogenic differentiation factor-1. |
| **NO** | Nitric oxide. |

| | |
|---|---|
| **NOS** | NO synthase. |
| **NPD** | Normal pellet diet. |
| **NPXpY** | NPX type of phosphorylated tyrosine. |
| **NT** | Nitrotyrosine. |
| **OD** | Optical density |
| **OGTT** | Oral glucose tolerance test |
| **OPD** | O-Phenylenediamine dihydrochloride**.** |
| **P** | Probability |
| **PAI-1** | Plasminogen activator inhibitor**.** |
| **PCI/3** | Prohormone convertase. |
| **PI3K** | Phosphoinositide-3 kinase . |
| **PKA** | Protein kinase A. |
| **PK**C | Protein kinase C. |
| **p38MAPKs** | p38mitogen-activated protein kinase**.** |
| PPARα | Peroxisome proliferator-activated receptor alfa |
| **PTB** | Poly pyrmiden tract binding domain. |
| **ROS** | Reactive oxygen species. |
| **SA** | Streptavidin**.** |
| **SCD** | Stearoyl-CoA desaturase. |
| **SHPTP$_2$** | Syrup-tyrosine-specific phosphatase. |
| **SREBP** | Sterol regulatory element binding protein. |
| **STE** | Sterol responsive elements. |
| **SU** | Sulfonylurea . |
| **TC** | Total cholesterol**.** |
| **TCF7L2** | Transcription factor gene variant. |
| **T2DM** | Type 2 diabetes mellitus**.** |
| **TG** | Triglycerides**.** |
| **TNF α** | Tumor necrosis factor α**.** |
| **UKPDS** | U.K. Prospective Diabetes Study . |
| **VDCC** | Voltage-dependent Ca2+ channels**.** |
| **VLDL** | VeryLow-density lipoprotein. |

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# ABSTRACT

Glucagon like peptide-1**(GLP-1**) is an incretin hormone which is responsible for insulin secretion in response to hyperglycemia .**GLP-1** is secreted from intestinal cells .Both high fat diet ,high fructose intake contribute to development of insulin resistance .

Rats used in the present study included normal control rats, high fat fed rats (**40% fat**) while the 3rd group received high fructose concentration in drinking water (**60% fructose**).Fasting blood samples were collected for the study of different parameters in fasting plasma, also post-prandial plasma for the study of **GLP-1** level, systolic blood pressure was measured in the 3 groups.

**Results:** The results of the present study was the development of insulin resistance with high fat diet, high fructose intake. In both insulin resistant groups, there was significant elevation of fasting plasma glucose, fasting plasma insulin, fasting plasma ( cholesterol, triglycerides and **LDL** levels ). On the contrary, there was highly significant reduction of post-prandial **GLP-1** and fasting plasma **HDL** levels in comparison with control group.

There was also rise of systolic blood pressure in insulin resistant rats. **It is concluded** that consumption of excess fat, high fructose intake in diet, play a role in increasing incidence of insulin resistance. Also the important finding of the reduction of post-prandial plasma **GLP-1** level in insulin resistant rats is suggestive for the use of **GLP-1** agonists or **dipeptidyl-peptidase-IV(DPP IV)** inhibitors as a line for treatment of type 2 diabetes mellitus.

**Key wards: GLP-1 ,Insulin resistance , Diabetes mellitus .**

# INTRODUCTION AND AIM OF THE WORK

Incretins are hormones released from the gastrointestinal tract in response to nutrient ingestion that potentiate glucose-stimulated insulin secretion from islet beta cells(**Creutzfeldt ,1979**). The 2 predominant incretins are glucagon-like peptide **(GLP)-1** and glucose-dependent insulinotropic peptide **(GIP).** These 2 peptides stimulate insulin secretion and unlike other insulinotropic agents, they do so in a glucose-dependent manner. In light of these beneficial actions, **GLP-1** and **GIP** represent potential therapeutic agents for the treatment of type 2 diabetes. However, exogenous **GIP** is comparatively less effective than **GLP-1** in stimulating insulin secretion in type 2 diabetes **(T2DM),** whereas the insulinotropic action of **GLP-1** is well preserved(**Holst et al., 1993**).So much of the current research has focused on enhancing **GLP-1** action for the treatment of type 2 diabetes.

**GLP-1** also exerts a number of other biological actions that contribute to its ability to lower glucose, including inhibition of gastric emptying, which reduces meal-associated increase in glycemic excursion. GLP-1 also inhibits glucagon secretion(**Komatsu et al., 1989)** and suppresses food intake in both diabetic and nondiabetic humans**(Gutzwiller et al .,1999)**. Furthermore, **GLP-1** has the potential to preserve or enhance beta-cell function in human subjects with type 2 diabetes due to its ability to stimulate beta-cell proliferation and neogenesis and inhibit apoptosis(**Brubaker &Drucker , 2004**).

The major therapeutic drawback in using native **GLP-1** is its very short half-life of less than 2 minutes following exogenous administration, due in part to the protease dipeptidyl peptidase **(DPP)-IV**(**Deacon et al., 1995**). As a result of preventing the degradation of native **GLP-1** through inhibiting the activity of the **DPP-IV** enzyme ,this offers a therapeutic strategy for enhancing endogenous **GLP-1** action in vivo.

**DPP-IV** is a ubiquitously expressed serine protease that exhibits postproline or alanine peptidase activity, thereby generating biologically inactive peptides via cleavage at the N-terminal region after *X*-proline or *X*-alanine**(Drucker, 2003).**Because both **GLP-1** and **GIP** have an alanine residue at position 2, they are substrates for **DPP-IV. DPP-IV** inhibitors are orally administered drugs that improve glycemic control by preventing the rapid degradation of incretin hormones, thereby resulting in post-prandial increase in levels of biologically active intact **GLP-1** and **GIP.**

Type 2 diabetes(**T2DM**) is characterized by a gradual progressive decline from near-absent first-phase glucose- induced insulin secretion to impaired second-phase insulin secretion, glucose potentiation, and disproportionate hyperproinsulinemia, with impaired basal or steady-state insulin secretion (**Kahn et al., 1994) .** Patients with clinical disease and fasting hyperglycemia are at the end stage of this process and demonstrate all of these features.

The remarkable finding is that hyperglycemia compensates for the impaired glucose potentiation and second-phase defect so that, at the intermediate stages of final β-cell failure (fasting plasma glucose <200 mg/dl), non glucose secretogogues are able to produce an insulin response that is absolutely normal in both magnitude and timing(**Pfeifer et al ., 1981**).

This response includes such diverse signals as **GLP1**, **secretin, the $\beta_2$-adrenergic agonist isoproterenol, tolbutamide, arginine, and other amino acids**. In a small number of studies performed some time ago, the impact of glycemic potentiation was very similar for all of these stimuli. Therefore, it is concluded that because we have found no data indicating otherwise, **the defect is attributed to an islet mechanism that is directly related to the unique way in which glucose regulates insulin secretion.**

## AIM OF THE WORK:

The aim of the study is to detect the effect of high fat diet &high fructose intake on inducing insulin resistance .Also the effect of insulin resistance on plasma lipid profile, plasma **GLP1** levels and systolic blood pressure.

# REVIEW OF LITERATURE

# INSULIN

## Synthesis and release of insulin:

Insulin is a hormone produced in the pancreas and released when any of the several stimuli is detected. The stimuli include ingested protein and glucose in the blood produced from digested food. Carbohydrates produce glucose, although not all types of carbohydrates produce glucose and thereby increase blood glucose levels. In target cells, they initiate a signal transduction, which has the effect of increasing glucose uptake and storage. Finally, insulin is degraded, terminating the response.

Insulin undergoes extensive post-translational modification along the production pathway. Production and secretion are largely independent; prepared insulin is stored awaiting secretion. Both C-peptide and mature insulin are biologically active. Cell components and proteins in this image are not to scale.

The endogenous production of insulin is regulated in several steps along the synthesis pathway:(At transcription from the insulin gene ,In messenger ribonucleic acid (**mRNA)** stability and at the **mRNA** translation In the posttranslational modifications).

It has been shown that insulin and its related proteins, are also produced inside the brain and that reduced levels of these proteins are linked to **Alzheimer's disease**. **(Gustin, 2005 ,de la Monte &Wands, 2005 and steen et al., 2005)**.

When the glucose level comes down to the usual physiologic value, insulin release from the beta cells slows or stops. If blood glucose levels drop lower than this, especially to dangerously low levels, release of hyperglycemic hormones(**most prominently glucagon from Islet of Langerhans' alpha cells**) causes release of glucose into the blood from cellular stores, primarily liver cell stores of glycogen. By increasing blood glucose, the hyperglycemic hormones prevent or correct life-threatening hypoglycemia. Release of insulin is strongly inhibited by the stress hormone **norepinephrine (noradrenaline)**, which leads to increased blood glucose levels during stress.

Evidence of impaired first phase insulin release can be seen in the glucose tolerance test, demonstrated by a substantially elevated blood glucose level at 30 minutes, a marked drop by 60 minutes, and a steady climb back to baseline levels over the following hourly time points.

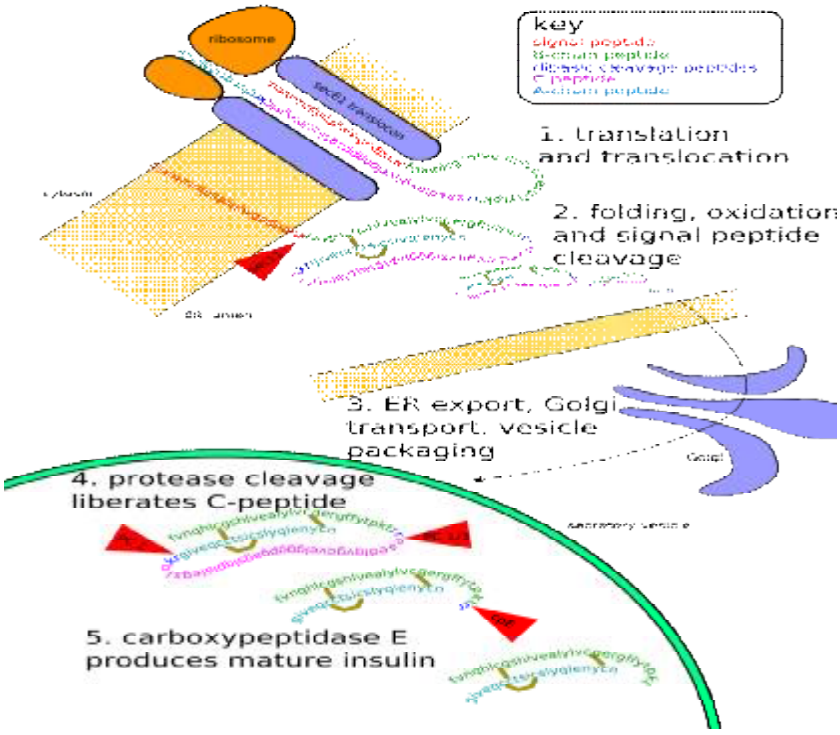


**Fig 1**.Insulin undergoes extensive posttranslational modification along the production pathway. Production and secretion are largely independent; prepared insulin is stored waiting secretion. Both C-peptide and mature insulin are biologically active. Cell components and proteins in this image are not to scale.