

Evaluating the need for a paradigm shift in the understanding of pathogenesis of type 2 diabetes

विद्या वाचस्पति की
उपाधि की अपेक्षाओं की आंशिक पूर्ति में प्रस्तुत शोध प्रबंध

A thesis
submitted in partial fulfilment of the requirements
of the degree of
**DOCTOR OF PHILOSOPHY
IN BIOLOGY**

द्वारा / By
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2024**

CERTIFICATE

This is to certify that the work incorporated in the thesis entitled “**Evaluating the need for a paradigm shift in the understanding of type 2 diabetes**” submitted by **Akanksha Ojha** was carried out by the candidate under my supervision. The work presented here or any part of it has not been included in any other thesis submitted previously for the award of any degree or diploma from any other university or institution.

Date: 27th June, 2023



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DECLARATION

I declare that this written submission represents my ideas in my own words and where others' ideas have been included; I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that the violation of the above will be the cause for disciplinary action by the institute and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

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Introduction

Earlier work from our lab has shown that type 2 diabetes mellitus (T2DM) progression is not majorly dependent on insulin-glucose deregulation. A network model by Dr. Shubhankar Kulkarni revealed that in absence of multi-organ network, impaired insulin signaling alone did not give a stable insulin resistance state. Further, the model predicted the reversal of T2DM state using behavioral and neuro-endocrine intervention, but normalization of glucose alone failed to do so (Kulkarni et al., 2017). Similarly, Dr. Manawa Diwekar-Joshi's work using multiple approaches showed that fasting glucose was independent of insulin regulation, although the postprandial curve was significantly affected (Diwekar-Joshi & Watve, 2020). The emerging view in the field supporting the above studies speculates the role of brain in regulating the insulin signaling and hyperglycemia. T2DM patients are shown to experience low brain glucose levels, with blunted brain glucose curves compared to normal healthy individuals (Hwang et al., 2017). T2DM is also known to affect vasculature, CVD (cardiovascular disease) being the most common complication of T2DM (Hu & Stampfer, 2003). A study by Hunt et al shows that subclinical atherosclerosis can predict future risk of type 2 diabetes (Hunt et al., 2003). A plausible theory gaining weight is that T2DM may be a result of vasculature changes, resulting in low glucose supply to brain and finally compensatory hyperglycemia induced by the brain. We planned to test this hypothesis using both experimental and theoretical means. The objectives of my study are listed below:

Specific objectives of the project:

To determine the role of brain in T2DM development in streptozotocin (STZ) induced diabetes mice models

Studies on STZ induced mice models have been central to glucose-insulin theory for T2DM. STZ induces hyperglycaemia by beta cell destruction, supporting the theory that impaired insulin signals are responsible for T2DM. But it is shown that beta cells recover quickly after STZ treatment, but hyperglycemia continues. This challenges the classical theory. My experiments show that not all STZ animals develop hyperglycemia and the hypoglycemia that develops within 6-12 hours of STZ treatment is a good predictor of later hyperglycemia. STZ is also known to affect FGF expression, vascularization of brain and reduced glucose transport to the brain so my hypothesis was that infusion of glucose to brain would reduce peripheral hyperglycemia.

Experiments on these lines could not be completed due to repeated pandemic lockdowns. So, I expanded the theoretical lines of work.

Historical analysis of the field to study the emergence of various hypotheses and examine the anomalies accumulating in the field

I analyze various evidences and anomalous findings in the field in perspective of two prominent philosophies of science, Karl Popper and Thomas Kuhn (Kuhn, 1970; Popper, n.d.). This part of the work examines which parts of the current T2DM paradigm are challenged by experiments, whether the current status of the field resembles the crisis state described by Kuhn, that precedes a paradigm shift and from Kuhn's analysis can a paradigm shift be predicted? The anomalies include multi-species variability across animal kingdom in the obesity-insulin-glucose relationship and why simple biochemical explanations don't work.

Collective analysis of clinical trial towards normalization of glucose in type 2 diabetes patients

The failure of glucose normalization treatment to arrest diabetic complications is also an anomaly but literature on it is contradictory. So, I did a meta-analysis of major non-pharma driven clinical trials. I also examined the scientific rigor of these trials. The emerging picture is that glucose normalization does not reduce the incidence of complications or mortality.

Development of an alternative paradigm model

I modelled a brain centered glucose homeostasis model to address the question whether the anomalies can be satisfactorily explained by the hypothesis that reduced glucose supply to the brain is the cause of fasting hyperglycemia. The model is used to test whether its predictions match with the anomalous empirical patterns qualitatively. The results suggest that this hypothesis works substantially better than the insulin resistance model. The model also predicts additional testable predictions (Ojha & Watve, 2022).

The historical, meta-analytical and modeling exercise converge to indicate that the insulin resistance centered view held currently has several anomalies pointing to a need for paradigm shift and the brain vasculature related causal model is a promising alternative paradigm.

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Popper, K. (n.d.). Karl Popper: The Logic of Scientific Discovery.



List of Publications

Research Articles

- Baig, U., **Ojha, A.**, & Watve, M. (2017). The evolving opportunistic pathogen communities on host individuals and the evolution of host aging. *Journal of Evolutionary Medicine*. <https://www.ashdin.com/abstract/the-evolving-opportunistic-pathogen-communities-on-host-individuals-and-the-evolution-of-host-aging-3236.html>
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- **Ojha, A.**, & Watve, M. (2018). Blind fish: An eye opener. *Evolution, Medicine, and Public Health*, 2018(1), 186–189. <https://doi.org/10.1093/emph/eoy020>
- Watve, M., & **Ojha, A.** (2022). The predictive value of Kuhn's anomaly and crisis: the case of type 2 diabetes. *Academia Letters*. <https://doi.org/10.20935/al5494>



List of Abbreviations

ACCORD	Action to Control Cardiovascular Risk in Diabetes
ADA	American Diabetes Association
ADVANCE	Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation
AMPK	AMP-Activated Protein Kinase
ATP	Adenosine triphosphate
ARH	Arcuate nucleus of hypothalamus
ARR	Absolute risk reduction
BBB	Blood brain barrier
BATIRKO	Brown adipose tissue insulin receptor knock-out
BDNF	Brain derived neurotropic factor
BMI	Body mass index
CB	Citrate buffer
CNT	Control
CI	Confidence interval
CNS	Central nervous system
CSS	Consequential steady state
df	Degrees of Freedom
DM	Diabetes mellitus
DPP	The Diabetes Prevention Program
DZX	Diazoxide



EM	Evolutionary medicine
Eq	Equation
ER	Endoplasmic reticulum
FDG-PET	Fluorodeoxyglucose-Positron emission tomography
FFA	Free fatty acid
FG	Fasting glucose
FGF	Fibroblast growth factor
FI	Fasting insulin
FIRKO	Fat insulin receptor knock-out
GDM	Gestational diabetes mellitus
GH	Growth hormone
GnRH	Gonadotropin-releasing hormone
GLUT	Glucose transporters
GTT	Glucose tolerance test
HCG	Human chorionic gonadotropin
HbA1c	Glycated haemoglobin
HOMA	Homeostatic model assessment
IAEC	Institutional animal ethics committee
ICV	Intracerebroventricular injections
IDE	Insulin degrading enzyme
IDF	International Diabetes Federation
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance



IGF	Insulin like growth factors
IMTG	Intramyocellular triacylglycerol
INSL	Insulin like peptides
IP	intra peritoneal
IPGTT	Intra peritoneal glucose tolerance test
IR	Insulin resistance
IRKO	Insulin receptor knock-out
IRS	Insulin receptor substrate
JH	Juvenile hormone
KO	Knock-out
LH	Luteal hormone
LHRH	Luteal hormone releasing hormone
LIRKO	Liver insulin receptor knock-out
MC4R	Melanocortin 4 receptor
MIRKO	Muscle insulin receptor knock-out
MODY	Maturity onset diabetes of the young
mTOR	Mechanistic/mammalian target of rapamycin
n or N	Sample size, number
ND	Non-diabetic
NFG	Normal fasting glucose
NSILA	Non-suppressible insulin like activity
NTNT	Number-time (person-years) of treatment needed
OGTT	Oral glucose tolerance test



ON	Overnight
OR	Odds ratio
POMC	Proopiomelanocortin
PS	Perturbed state
r	Pearson's correlation coefficient
rho	Spearman's correlation coefficient
R ²	R-squared
RCT	Randomized controlled trial
RECORD	Rosiglitazone evaluated for cardiovascular outcomes
RLN	Relaxin
S6K1	Ribosomal protein S6 kinase beta-1
SE	Standard error
SGLT2	Sodium glucose co-transporter 2
SS	Steady state
STZ	Streptozotocin
T1D	Type 1 diabetes
T1DM	Type 1 diabetes mellitus
T2D	Type 2 diabetes
T2DM	Type 2 diabetes mellitus
TSS	Targeted steady state
TSH	Thyroid-stimulating hormone
UGDP	University Group Diabetes Program
UKPDS	United Kingdom Prospective Diabetes Study



VADT	Veterans Affairs Diabetes Trial
WHO	World Health Organisation
β IRKO	β -cell insulin receptor knock-out



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CHAPTER 1

Introduction



1.1 Type 2 Diabetes: Pathogenesis and aetiology

1.1.1 What is the classical theory of diabetes?

According to International Federation of Diabetes, type 2 diabetes is 90% of all diabetes cases and is one of the most common metabolic disorder. In 2021, the global number of diabetic patients was approximately 537 million adults, which is estimated to increase to 643 million by 2030 and 783 million by 2045 (IDF facts and figures).

Type 2 diabetes is commonly defined as a metabolic syndrome characterized by hyperglycemia, insulin resistance, low glucose clearance rate, with advanced stages involving neuropathy, retinopathy, nephropathy and cardiopathy.

The classical theory of development of type 2 diabetes states that a sedentary lifestyle with high calorie intake leads to obesity and subsequently insulin resistance via complex mechanisms, leading to lower absorption of glucose and hyperglycemia, which further induces insulin secretion by pancreas. As a result in a transient phase, there is hyperinsulinemic but normoglycemic phase. This increases pressure on pancreatic beta cells, leading to dysfunction as well as reduction in their number. This feeds back into lower glucose clearance and further high plasma glucose. It is this high levels of glucose which is theorized to lead to organ system decay due to glycation and other mechanisms, finally leading to morbid condition (DeFronzo et al., 2015).

1.1.2 How does classical theory explain the origin of insulin resistance?

Classical theory of diabetes does not specifically commit on the causal origin of type 2 diabetes. It always hinges on the manifested symptoms of the disease. Since most of the research and data is based on western population, the primary cause of insulin resistance in T2DM is accepted to be obesity. It is stipulated that increased glucose intake with increased FFA circulation and changed incretin action leads to insulin resistance. The molecular mechanism of insulin resistance involves phosphorylation of serine residues on IRS (Insulin receptor substrate), such that it can no longer be activated downstream of insulin binding to receptor. Multiple independent factors like, pro inflammatory cells, increased lipid molecules, mTor signalling have been shown to affect the phosphorylation of IRS leading to insulin resistance (Andreas et al., 2000; Hardy et al., 2012; Xu et al., 2003). But unlike the classical assumption, insulin resistance itself is preceded by hyperinsulinemia, which is increasingly getting



acceptance in the field (Abdul-Ghani & DeFronzo, 2021; Dana, 1954; DeFronzo et al., 2015). This observation leads to the possibility that hyperinsulinemia is causal to insulin resistance (M. Watve, 2012). As yet there is little clarity about the exact cause of insulin resistance and whether IRS related molecular mechanisms are sufficient to account for the pathophysiological effects.

1.1.2 What are different observations which are difficult to explain by the classical theory of glucose homeostasis?

As we have discussed earlier that although we understand major symptoms of type 2 diabetes, the origin is still shrouded with mystery. The discovery of insulin played a major role in changing the direction of research on diabetes. Isolation of insulin and its administration proved a miracle for Type 1 diabetes, which was the most common form of diabetes before 1920's. This led to an explosion of research on control of plasma glucose and effects of insulin on glucose homeostasis. It led to an understanding of insulin centric glucose homeostasis in *Homo sapiens*. The classical theory has since been modified to accommodate many different observations that were inexplicable originally. But overtime, accommodating all observations has become difficult, without entirely modifying the classical theory of type 2 diabetes mellitus. Experimental and epidemiological data on effect of glucose normalization on mortality and morbidity, effect of tissue specific insulin receptor knockout on peripheral glucose levels, effect of suppressing insulin degradation enzyme on glucose levels, ICV glucose infusions, ICV insulin infusions, contradict the expectations of the classical theory (Diwekar-Joshi & Watve, 2020).

1.2 Functional evolution of Insulin and related proteins

1.2.1 Discovery of insulin

The time before 1922 in the field of diabetes is described as the “Frustration period” by Arthur Colwell. This era was defined by high rate of mortality, acidosis, high infection incidences and bizarre diet practices amongst the diabetic population (Colwell, 1968; Vecchio et al., 2018). The term “insuline” was coined by deMayer in 1909, to describe the active agent found in pancreatic extracts (Bliss, 2021; MacLeod, 1978). There is a history of multiple failed attempts by various research groups to create an active pancreatic extract which could be used for therapeutic purpose. Vecchio et al (2018) comprehensively illustrates efforts by various groups throughout history from discovering the anatomy of pancreas to alcohol based extraction of islets of Langerhans, all of which were vital for the prominent work by Banting, Best and



Noble. Most of the researchers faced difficulty on isolating the islets from the pancreatic exocrine tissue. Banting developed a technique where closing the pancreatic duct inside the animal lead to degeneration of exocrine tissue and not the islets, thus giving them isolated islets (Banting et al., 1922). Later Best with help of James Collip established acid alcohol extraction method from whole pancreas. The above extracts gave positive results in experimental, depancreatized dogs, and were authorized to be tested in humans, where they were shown to normalize, glycemia, glucosuria and ketonuria (Banting, 1929; Banting et al., 1923; Bliss et al., 1982; Vecchio et al., 2018). Thus began the unified journey of insulin and diabetes.

1.2.2 Insulin family of proteins

Insulin became a model for studying structure and functions of proteins. It was the first protein molecule to be sequenced in 1955 by Fredrick Sanger. It also became the first protein to be completely chemically synthesized in 1968, by Helmut Zahn's group and its three dimensional structure solved in 1969 by Dorothy Hodgkins. As the era of genomics flourished, many insulin like sequences were discovered across the species. In addition to the insulin gene (INS) in vertebrates, further nine insulin-like peptides were discovered, which are now classified as insulin superfamily. The insulin superfamily consists of insulin, two insulin-like growth factors (IGFI and IGFI) and three members of the relaxin-like peptide family (RLN1, RLN2, and RLN3) and four insulin-like peptides (INSL3, INSL4, INSL5, INSL6) (Adham et al., 1993; Bathgate et al., 2002; Chassin et al., 1995; Conklin et al., 1999; Hudson et al., 1983, 1984; Lok et al., 2000).

Insulin

As we know from the story of discovery of insulin that it was primarily isolated for its property of regulating blood glucose levels. It is now known that insulin increases glucose uptake in muscles, liver and adipose tissue, by activating receptor tyrosine kinase, which further acts on insulin receptor substrates (IRS), increasing the expression of glucose transporters on cell membrane of the tissue, leading to increased glucose uptake. At the same time, insulin also suppresses mobilization of fuels by inhibiting the breakdown of glycogen in the liver, the release of amino acids from muscle and the release of free fatty acids from adipose tissue



(Mayer et al., 2007; Tokarz et al., 2018). Biologically active form of insulin is a monomer

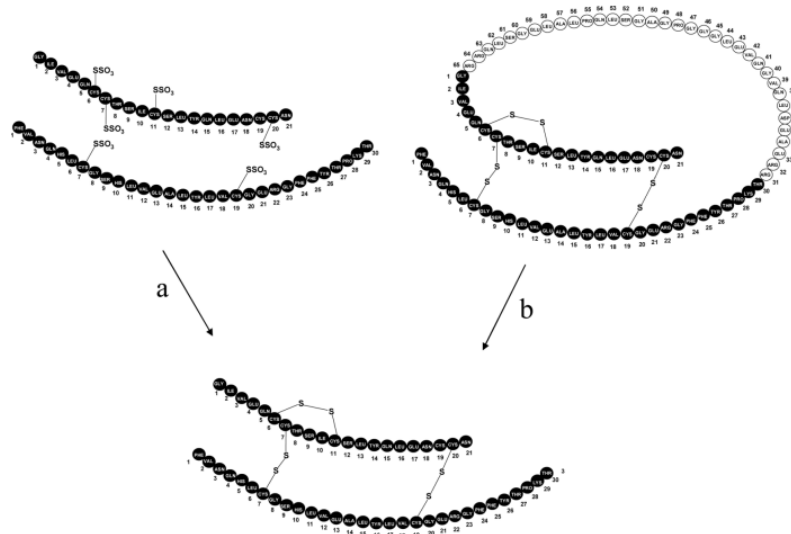


Figure 1.1: An adaption of insulin structure. (Mayer et al 2007)

consisting of two chains, an A chain of 21 amino acids and a B chain of 30 amino acids linked by two disulfide bridges (Figure 1.1).

Insulin Like Growth Factors (IGF-I and IGF-II)

Insulin like growth factor-I (IGF-I) was identified in human serum in 1963, as a peptide with insulin like properties but not suppressed by anti-insulin antibody hence was initially named NSILA (Non-suppressible insulin like activity). IGF-1 was found to be a single chain peptide with 70 amino acid. Both growth hormone (GH) by pituitary gland and insulin are known to induce IGF-1 production. IGF1 production is mainly done by the liver and also in several tissues including the brain, testes, skeletal muscle, bone, and cartilage. In 1976, a similar peptide was sequenced and was identified as insulin like growth factor-2 (IGF-2). Both these peptides were found to be similar in structure to proinsulin and were found to responsible for cell proliferation, differentiation and maintenance. Although both have similar functions, their expressions are temporally differentiated. IGF-2 is expressed and involved in placenta development and fetal growth whereas IGF-1 is expressed postnatally under the activation of growth hormone (GH) to regulate growth and development throughout life (Bowman et al., 2010; Hakuno & Takahashi, 2018; Randhawa, 2008; Shabanpoor et al., 2009; Wang, 1991).

Relaxin Like Peptides (RLN1, RLN2, RLN3)



The first relaxin family proteins were discovered in 1983 and 1984, in great apes, and were called relaxin-1 and relaxin-2. They were named relaxin, because of their ability to relax the pelvic ligaments. Although discovered later, it is relaxin-2 peptide which is the major form of relaxin circulating and functional equivalent of relaxin-1 in *Homo sapiens*. Relaxin-3 was discovered when looking for insulin like peptides in human genome (Bathgate et al., 2002). All relaxin like peptides show 50% similarity to insulin sequence and show similar structure containing two peptides A and B connected by two disulphide bridges. It is postulated that relaxin and insulin evolved from a similar ancestral gene and are hence clustered in same family of proteins (Wilkinson & Bathgate, 2007). Relaxin-2 has been shown to play vital role in vertebrate reproduction and growth. Relaxin knockout female mice have exhibited prolonged labour and inability to deliver litter normally (Samuel et al., 2007; Zhao et al., 1999). In female humans, relaxin concentration increases to its maximum from conception to first trimester and then remains stable throughout pregnancy (AgoulNIK, 2008; Bell et al., 1987; Eddie et al., 1986). Relaxin is also postulated to play a role in early follicular development and embryo implantation in humans (Park et al., 2005). Relaxin-2 is also shown to have anti-inflammatory effect and promote wound healing. These properties of relaxin-2 has made it an attractive therapeutic agent for allergic responses, cardiac protection, fibrosis, wound healing and cancer (Samuel et al., 2007).

Insulin Like Peptides (INSL3, INSL4, INSL5, INSL6)

INSL3 was discovered in 1990 as a part of differential screening of porcine testis specific transcripts, hence was initially called as Leydig insulin like peptide. INSL3 and relaxin are very similar in sequence, particularly the B chain. INSL3 knockout male mice exhibit retardation in testicular descent, whereas female knockout mice show impaired fertility with abnormal estrous cycle. Luteal hormone (LH) released by pituitary has been shown to increase the expression of INSL3 in Leydig cells (Adham et al., 1993). Although physiological effects of INSL3 are known, the specific bio signalling pathway it activates in the cell remains to be explored.

INSL4 was discovered in 1995 while screening early human placenta transcripts. It is expressed more strongly in the differentiated syncytiotrophoblast than in cytotrophoblast cells (Chassin et al., 1995; Laurent et al., 1998). It has also been detected in amniotic fluid and maternal serum during normal pregnancy (Mock et al., 1999), and in higher levels during pregnancies with trisomy (Millar et al., 2005; Mock et al., 2000, 1999). Very little is known about the biological



function of INSL4 peptide although it has been shown to be able to stimulate tyrosine phosphorylation, DNA synthesis and human chorionic gonadotropin (HCG) production which may suggest that INSL4 may be involved in cellular proliferation and differentiation during placental development (Koman et al., 1996).

INSL5 was discovered by two independent groups in EST databases based on sequence similarity with the A and B domains of insulin and relaxin. Highest expression of INSL5 in humans is in the colon. The function of INSL5 is still unknown; its expression profile suggests a nonreproductive role. INSL6 is the latest member of insulin superfamily which has been identified as a result of searching EST databases for proteins containing the insulin family B-chain cysteine motif (Lok et al., 2000).

1.2.3 Role of insulin in glucose regulation

Insulin was discovered for its role as a glucose lowering agent. As discussed earlier in the chapter the major targets for anabolic actions of insulin are liver, muscle and adipose tissue. At cellular level these cells express insulin receptor on their cell surface. In contrast to muscle and adipose tissue, insulin does not affect the liver specific glucose transporters (GLUT2) expression, hence has no effect on glucose uptake by liver. But it increases glycogen synthesis by stimulating glycogen synthetase and inhibiting glycogen phosphorylase. In muscles and fat tissue it induces rapid intake of glucose by increasing recruitment of intracellular GLUT4 on cell surface. Insulin reduces fat mobilization from adipocytes by inhibiting hormone-sensitive tissue lipase and stimulating re-esterification of FFAs. When the concentration of insulin decreases gradually the FFA's are released from the adipocytes in circulation. Further when insulin concentration increases it reduces formation of ketone bodies by inhibiting beta oxidation of FFA's (Bock & Goode, 2008; Mayer et al., 2007; Srivastava & Posner, 2012). Insulin also promotes uptake of essential amino acids into muscles and stimulates protein synthesis. Simultaneously, it prevents breakdown of protein and release of amino acids from muscle.

1.2.4 What is the primary function of insulin?

Although insulin is mainly perceived as a glucose lowering agent, over the 100 years of its discovery, there is large body evidence for its non-metabolic functions.

Most members of the insulin superfamily as described earlier have been shown to be part of growth and development and play important role in life strategy decisions. Initial studies of



insulin's effect on glucose metabolism in brain led to discovery of its wide effect of on energy regulation via behavioural modifications. Insulin after its discovery in 1920's for the longest of time assumed to have functions limited to the peripheral system. Its presence in rat brain discovered by Havrankova in 1978 led to a whole field of research to understand its effect on central nervous system. They also showed that severe reduction or elevation in peripheral insulin has little effect on the insulin concentration in the brain (Havrankova, Roth, et al., 1978; Havrankova, Schmechel, et al., 1978). The origin of insulin in brain has been disputed for a long time, but there is general consensus that pancreas is the major manufacturer and brain cells accumulate insulin transported through blood brain barrier (Ghasemi et al., 2013). In 1979, Woods et al, showed that chronic ICV infusion of insulin in lateral ventricles in baboons led to decrease in food intake and body weight in animals. Chronic infusion of insulin in other species and acute infusion of insulin also yielded similar results (Woods et al., 1979; Yavropoulou et al., 2009). It is now known that energy homeostasis and reproductive functions are interrelated (Smith et al., 2010; Wolfe et al., 2014). Arias et al (1992) showed that a low level of insulin stimulates the LHRH secretion. They also reported that stimulatory effects of insulin are dependent on the availability of glucose, while acute elevation of glucose level does not affect LHRH release (Arias et al., 1992). Further, Dong et al. showed that insulin loss in diabetic rats not only decreases gonadotropin-releasing hormone (GnRH) release from the hypothalamus, but also in the absence of insulin, the responsiveness of pituitary LH-releasing cells to GnRH is also decreased.

Insulin like peptides and insulin growth factors have been shown to play decisive role in life history mode selection in *Caenorhabditis elegans*, the fly *Drosophila melanogaster*, and the mouse *Mus musculus*. In *C. elegans*, the IIS pathway (Insulin and Insulin like growth factor signalling) is activated in presence of food or other environmental cues, which binds with DAF-2/insulin receptor in target tissues, leading to production of secondary endocrine hormones that promote reproductive mode. Similarly in *Drosophila*, activation of insulin or insulin like peptide signalling pathway leads to production of juvenile hormone (JH) by corpora allata, which leads to suppression of response to external stress but selection of reproductive mode. In mouse, food intake leads to activation of hypothalamus via neuronal input, increasing release of gonadotropins [luteinizing hormone (LH) and follicle-stimulating hormone (FSH)], thyroid-stimulating hormone (TSH), and growth hormone (GH) from the pituitary, but also increases insulin secretion which leads to increase in LHRH release (Tatar et al., 2003). Further longevity is shown to increase by 18% on disruption of fat specific insulin receptor genes in mouse



(Blüher et al., 2003). Insulin signalling pathway is implicated to be crucial in life history mode selection (Pan & Finkel, 2017).

Insulin has also been shown to effect cognition by Benedict et al, who have shown that prolonged increase in CNS insulin by 8-week intranasal administration improved memory and mood in Alzheimer's disease patients. Insulin has been shown to have neuroprotective effects in the brain. It promotes neuronal survival, regulates neurotransmitter release, and modulates synaptic plasticity. Insulin receptors are present in various regions of the brain, and studies have suggested that insulin signalling plays a role in cognition, memory, and neurodegenerative disorders (Cholerton et al., 2012; Craft et al., 2012; Duarte et al., 2012). Insulin also effects vascular function and plays a role in endothelial cell function, vascular tone regulation, and blood flow regulation. It helps to maintain the integrity and function of blood vessels. Impaired insulin signalling in endothelial cells has been linked to cardiovascular diseases (Kubota et al., 2013; Rask-Madsen & King, 2013).

Based on the variety of functions that insulin is known to carry out but also its evolutionary history of evolving from a common ancestor with IGF-1 like function, glucose metabolism may not be its primary purpose. Insulin functions seems to diverge in peripheral and central systems leading us to investigate its role in development of type 2 diabetes.

1.3 Obesity, Insulin Resistance and Insulin Response

Obesity, characterized by excess body fat accumulation, is a major risk factor for the development of insulin resistance and type 2 diabetes. Adipose tissue, particularly visceral adipose tissue, secretes pro-inflammatory cytokines, adipokines, and free fatty acids, which can impair insulin signalling and promote insulin resistance. Although obesity is seen as risk factor for developing type 2 diabetes, most of the obese people do not become diabetic.

The relationship between obesity and insulin resistance is complex and involves multiple mechanisms. While the exact mechanisms are not fully understood, several factors contribute to the development of insulin resistance in the context of obesity.

In obesity, adipose tissue undergoes significant changes, particularly in visceral adipose tissue, which is more metabolically active. Increased adiposity leads to adipose tissue dysfunction, characterized by altered adipokine secretion, increased release of free fatty acids (FFAs), and chronic low-grade inflammation (Björntorp, 1991). Excessive fat accumulation in non-adipose tissues, such as liver, muscle, and pancreas, is associated with insulin resistance. This ectopic fat deposition is believed to impede normal cellular processes, impair insulin signalling, and



disrupt glucose and lipid metabolism (Boden, 2011; Larson-Meyer et al., 2011; Xu et al., 2003; Yazıcı & Sezer, 2017). Obesity is characterized by a state of chronic low-grade inflammation. Adipose tissue produces pro-inflammatory cytokines such as tumour necrosis factor-alpha, interleukin-6 and chemokines that can interfere with insulin signalling pathways (Tilg & Hotamisligil, 2006). Elevated levels of circulating FFAs, a characteristic of obesity, can lead to lipotoxicity. Excessive FFAs can accumulate in non-adipose tissues, such as skeletal muscle and liver, and interfere with insulin signalling and glucose metabolism. FFAs can also induce cellular stress, mitochondrial dysfunction, and inflammation, further promoting insulin resistance. Adipose tissue secretes various hormones and adipokines that regulate metabolism. In obesity, there is dysregulation in the secretion of these hormones, such as adiponectin, resistin, and leptin. Altered levels of these hormones can contribute to insulin resistance and disrupt normal metabolic processes (Samuel, V. T., & Shulman, G. I. 2018).

Although a lot of associations have been observed between various obesity related factor and insulin resistance, little clarity exists to mechanistically link causation to any of these associations. Adipose tissue is an important endocrine tissue involved intricately in energy homeostasis with heavy innervation from the central nervous system (Hui, 2018; Kosicka, 2014; Martinez-Sanchez et al., 2022). It is only recent that disrupted metabolic physiology observed in obese patients is now being studied as a neuroendocrine reprogramming (Ghanemi et al., 2021).

1.4 Glucose-insulin regulatory mechanisms

During the 19th century, experiments conducted by Claude Bernard demonstrated that fourth ventricle of the brain was capable of regulating peripheral glucose levels. By 20th century the focus had shifted to understanding the role of pancreas in energy homeostasis. With the discovery of insulin as a glucose regulating agent in 1922, the focus shifted completely to the peripheral glucose regulation, while ignoring central glucose regulation. Our current understanding of central glucose regulation is a result of research conducted in last two decades. This research has not yet found an appropriate place in clinical thinking. Also as yet we don't understand why two different mechanisms have evolved and how they interact with each other contextually.

1.4.1 Peripheral mechanism of glucose regulation



The peripheral mechanism of glucose regulation includes hormone dependent glucose uptake or release in the blood stream. Pancreas, liver, muscles and adipose tissues play major role in peripheral glucose regulation. The alpha and beta endocrine cells in islet of Langerhans in pancreas produce insulin and glucagon respectively. When the blood glucose levels increase after meal, the insulin released by beta cells signals liver, muscles and adipose tissue to increase glucose uptake. Similarly, in starvation when blood glucose levels drop below a certain threshold (4-6 mM), glucagon released by alpha cells signals liver, muscles and kidneys to produce glucose via glycogenolysis and gluconeogenesis (Lin et al., 2021; Ruud et al., 2017). There is an array of studies describing the mechanisms via which glucose and free fatty acids induce insulin release, where the most commonly accepted models is based on work by Dean and Matthews (Dean & Matthews, 1968, 1970).

The model proposes that upon increased uptake of glucose via cell membrane and glucose transporters (GLUT), the glycolysis and subsequent utilization in tricarboxylic acid cycle leads to increase in ATP in the beta cells. This increase in ATP concentration leads to closing of ATP-sensitive K channels, leading to depolarization of cell membrane. This depolarization further induces opening L-type voltage dependent Ca^{2+} channels, leading to increased exocytosis of insulin containing vesicles (Komatsu et al., 2013). Further theoretical and empirical work has revealed details of this mechanism including, pulsating nature of insulin release from beta cells and different mechanisms for lipid induced or peptide induced release of insulin (Bergsten, 2000; Gilon et al., 2002; Grodsky, 1972; Maechler et al., 2006; Matthews et al., 1983; Pørksen, 2002; Saltiel & Pessin, 2007).

1.4.2 Central mechanism of glucose regulation

Pioneering experiments by Claude Bernard in 19th century demonstrated that damage to the fourth ventricle in the brain could lead to an increase in glucose levels in experimental animals, which was excreted as glucose in urine (Holmes, 1974; Noble, 2008). But the field remained unexplored until recently. There is now rapid accumulation of evidence indicating a primary role of the central nervous system in regulation of glucose and insulin levels. It is now known that the pancreas, liver and adipose tissue are heavily innervated by autonomic nervous system. The islets of Langerhans are innervated richly by sympathetic fibres, parasympathetic fibres as well as nociceptive sensory fibres (Bovara et al., 2002; Lin et al., 2021). The sympathetic innervation in pancreas is mediated via splanchnic nerves. The study of specific effect of



sympathetic innervation on peripheral organ function has been challenging work, but it has shown that electrical stimulation of splanchnic nerve in the pancreas, leads to decrease in GSIS (glucose stimulated insulin secretion) and increase in glucagon secretion. This effect is synonymous with the activation of sympathetic system in human body in case of starvation or hypoglycemia. But the effect of sympathetic innervation on other islet hormones is not well understood (Patel, 1984; Taborsky & Munding, 2012).

The parasympathetic innervation of pancreas originates from the dorsal motor nucleus of vagus (Ahrén, 2000; Lin et al., 2021). Stimulation of vagal nerve has shown increased secretion of both insulin and glucagon. Parasympathetic innervation is also implicated to regulate Beta cell proliferation (Moullé et al., 2019).

Sensory nerves innervating adipose tissue has been shown to be crucial in relaying information to CNS regarding adiposity as well as for regulating functions of adipocytes (Frei et al., 2022; Garretson, 2016; Guilherme et al., 2019; Murphy et al., 2013).

Aside from regulating the peripheral glucose, the CNS also regulates feeding habits, peripheral insulin sensitivity and regulating energy fluxes. The neurons of arcuate nucleus of hypothalamus (ARH) located at the floor of third ventricle are involved in sensing peripheral substances that signal energy state of the organism. POMC and AgRP/NPY secreted by two distinct population in ARH play vital role in energy homeostasis by affecting appetite and feeding behaviour. AgRP neuron activation has shown to reduce insulin stimulated uptake of glucose decreased by half selectively in brown adipose tissue (BAT) (Steculorum et al., 2016).

The classical theory believes that central mechanisms are important only to avoid hypoglycaemia. A counter regulatory response is launched by brain when glucose levels fall below a range, via ventromedial nucleus of hypothalamus (VMH). Activation of VMH neurons has been shown to elevate circulating levels of glucose and glucagon, and also lead to increased expression of gluconeogenic genes in liver (Stanley et al., 2016). A much larger body of work exists describing the molecular details of how the neurons in these specific regions of brain are activated under different physiological conditions (Levin et al., 1999; Parton et al., 2007; Routh, 2010; Shimazu & Minokoshi, 2017).



Logically coherent interpretation of the coexistence of the two systems can be potentially attempted on the platform of evolutionary physiology. Such attempts are only recent and I will make use of these concepts in this thesis.

1.5 Familial diabetes and genetics:

While a plethora of data highlights variations in the prevalence of type 2 diabetes among diverse ethnic populations, certain studies propose that socioeconomic factors may eclipse genetic influences as the primary causative element (Gardner et al. 1984; Knowler et al. 1988; J. C. Florez et al. 2009; Campbell et al. 2012; Cheng et al. 2012; Golden et al. 2019). Limited evidence suggests that dissimilarities in the frequencies of known susceptibility genetic alleles for type 2 diabetes do not consistently account for racial/ethnic disparities, with only a handful exhibiting such correlations (Hanson et al. 2015; Klimentidis et al. 2011). The exploration of genetic susceptibility has been uneven across global ethnic groups, but a recent study employing single-variant resolution mapping has identified 200 genetic variants consistently associated with type 2 diabetes across diverse ethnicities (Mahajan et al. 2018). While most variants exert small effects on diabetes risk, a subset, albeit rare in most populations, exerts significant influence. Notably, certain rare variants are more prevalent in specific, relatively isolated populations, elucidating a substantial portion of the excess risk in these groups, exemplified by the HNF1A variant in Oji-Cree Indians and a TBC1D4 variant in Inuit individuals (Hegele et al. 2003; Moltke et al. 2014).

Beyond genetic considerations, cultural behavioural factors, socioeconomic conditions and epigenetic factors may contribute to familial diabetes. The modal activities during leisure time vary between cultures. In cultures where leisure time is spent more in relaxing and comfort activities will have more incidences of diabetes than a culture where leisure time is spent more in swimming, biking, traveling, hiking, adventure activities etc. Anecdotally this cultural difference is remarkable, but there has been little systematic data collection on it. Clinical, epidemiological, and recent experimental evidence underscores the impact of environmental factors on the natural history of type 2 diabetes. Shared environments within family units have been identified as capable of inducing epigenetic changes, thereby modifying the function of genes associated with key diabetes traits. Epigenetic mechanisms emerge as plausible explanations for the environmental origins and familial aggregation of type 2 diabetes, often surpassing the explanatory power of common polymorphisms. Parental exposure to environmental factors known to induce epigenetic changes has been shown to reprogram



insulin sensitivity and beta-cell function in subsequent generations, indicating transgenerational transmission of specific epigenetic alterations. Noteworthy studies demonstrate that lifestyle interventions in humans can modulate the epigenome, reversing environmentally induced epigenetic modifications at specific target genes. This intricate interplay between genetic, environmental, and epigenetic factors underscores the multifaceted nature of type 2 diabetes etiology.

The most common flaw in all GWAS studies is that they define diabetes by the glucose levels and not by the pathophysiology of complications, the two being poorly correlated. Even if we ignore this flaw, GWAS performance is poor in terms of population variance explained.

Numerous studies have explored the genetic underpinnings of type 2 diabetes (T2DM). However, the identification of a common variant universally associated with T2DM across diverse populations remains elusive, as the genetic architecture of T2DM appears to involve hundreds of variants with modest effects (Albrechtsen et al. 2013; Steinthorsdottir et al. 2014; Mahajan et al. 2018; Kreienkamp et al. 2023; Xue et al. 2018; Jose C. Florez, Udler, and Hanson 2021; Wheeler and Barroso 2011). Notably, variants with stronger effects are either rare or unique to specific populations, complicating the establishment of broad genetic determinants (Knowler et al. 1988; Cai et al. 2020; Steinthorsdottir et al. 2014; Dallali et al. 2023; Moltke et al. 2014; Hanson et al. 2015). Genome-wide association studies (GWAS) primarily identify correlated single nucleotide polymorphisms (SNPs) without pinpointing causal variants, and the transition from association to molecular function is challenging. This challenge is particularly pronounced for non-coding regulatory variants, requiring distinct investigative approaches.

Despite the extensive efforts in GWAS and whole-genome sequencing, establishing a functional link between common genetic variants in T2DM patients and their contribution to blood-brain barrier (BBB) or vasculature function necessitates a targeted investigation. The complexity of T2DM etiology involves intricate interactions among multiple genetic and environmental factors. The surge in T2DM incidence cannot be solely attributed to genetics, as most GWAS are designed to detect loci with primary effects on T2DM risk, potentially missing variants whose impact varies in different environmental contexts (Jose C. Florez, Udler, and Hanson 2021).

Although comprehensive genomic studies have yet to yield concrete variants associated with T2DM, they lay the groundwork for profiling the predictive power of a genotype risk score (GRS) in conjunction with diverse environmental interactions. Moreover, insights from studies



such as The Diabetes Prevention Program reveal that intensive lifestyle interventions, encompassing dietary and physical activity components, can effectively mitigate T2DM risk even in individuals with a high load of known genetic risk variants (Hivert et al. 2011). This evidence instils optimism that T2DM, despite genetic susceptibility, can be ameliorated through targeted interventions and lifestyle modifications. Thus, the focus on non-genetic risk factors remains crucial in comprehensively understanding and addressing the complex landscape of T2DM pathology.

1.6 The burden of history:

The discovery of insulin as a glycolytic agent revolutionized the treatment of type 1 diabetes, providing significant benefits to patients. However, at the time of its discovery, there was no clear mechanistic distinction between type 1 diabetes (juvenile diabetes) and type 2 diabetes (adult-onset diabetes). Moreover, the majority of diabetes cases were of type 1, affecting children and young adults. Prior to 1922, a diagnosis of type 1 diabetes meant a drastically reduced lifespan, with frequent hospitalizations and the need for strict diets to control acidosis and ketosis. None of the available methods could guarantee an extended lifespan. Therefore, the discovery of insulin was undoubtedly a tremendous advancement.

However, alongside the discovery of insulin, it became evident that insulin was not effective in treating adult-onset diabetes, which was consequently termed non-insulin dependent diabetes mellitus (type 2 diabetes). Nonetheless, due to insulin being the only accepted glucose regulating agent, its use remained widespread. Despite severe side effects observed in type 2 diabetic patients, insulin and insulin resistance continued to be the focus of primary treatment prescribed for type 2 diabetes. However, these approaches, although reducing blood glucose levels, did not improve mortality rates in patients consistently across clinical trials. Despite the limitations of the insulin centric approach to treatment of T2DM, the clinical thinking has not changed.

While early studies on energy homeostasis hinted at the role of the central nervous system (CNS) in glucose regulation, the discovery of insulin resulted in a neglect of the CNS's glucoregulatory role for almost five decades. It is only recently that central glucoregulatory mechanisms in relation to diabetes and obesity have gained significant attention.

1.7 Previous work from our group and the objectives of my thesis:



Since 2007 the Watve lab has independently or in collaboration with other research groups has consistently pointed out the anomalies in the classical theory on the one hand and attempted to raise an alternative picture on the other. Our group showed the inconsistencies in the existing hypotheses about the origin of obesity and the relationship between obesity and insulin resistance (Vidwans and Watve 2017; Baig et al. 2019). Kulkarni et al (2017), using a network model challenged the central role of glucose and insulin in the pathophysiology of T2DM (Kulkarni, Sharda, and Watve 2017). Further Diwekar-Joshi and Watve (2020) challenged the insulin centered model of glucose regulation using convergence of multiple lines of evidence. An alternative view started emerging with Watve and Yajnik (2007) suggesting a behaviour based etiology of T2DM followed by a series of publications addressing the details of it (M. G. Watve and Yajnik 2007; M. Watve and Mandani 2008; Mankar et al. 2008; Belsare et al. 2010; Joshi et al. 2010; Baig et al. 2011, 2019; Karve et al. 2011; Patil et al. 2021; M. Watve, Bodas, and Diwekar 2014; M. Watve and Diwekar-Joshi 2016).

Since the pathophysiology of T2DM is complex, many gaps still exist in the emerging alternative view. My thesis focuses on addressing some of the gap areas and developing experimental, epidemiological and/or theoretical work to address the gap areas as a part of the ongoing journey towards a possible paradigm shift. I will address the following issues in the thesis

- Insulin was discovered in the context of type 1 diabetes and therefore by the burden of history became known as the glucose regulating mechanism. But eventually many different functions of the insulin and insulin like proteins became known. I briefly try to examine this ask the question is there any coherent theme in the functional evolution of insulin and related molecules. Is glucose regulation the centre of this evolution? In humans, is glucose regulation the main function of insulin?
- If the phenomena of obesity, insulin resistance, hyperinsulinemia etc were related by simple biochemical mechanisms such as FFAs induce insulin resistance, then these relationships would have been more or less similar in different species. I reviewed therefore the relationships among different species to find that they are different and often contradicting in different species. This indicates that generalized biochemical mechanisms are inadequate to explain these relationships. They appear to have evolved differently in different species and therefore we need to go beyond biochemical explanations and address the question why they evolved differently in different species and by which mechanisms.



- One of the backbones of the insulin centered view of diabetes is the streptozotocin (STZ) model of diabetes in rodents. STZ is shown to destroy the insulin producing beta cells. However, many other changes also happen in the body after STZ treatment. The beta cell destruction theory has been accepted without considering alternative causal pathways. I re-examine the STZ model experimentally as a potential challenge to the classical interpretation.
- The gluco-centric model of T2DM assumes that although the mechanism of deregulation of glucose in type 1 and type 2 diabetes is different, all further pathologies are driven by hyperglycemia and therefore regulating glucose is the focus of treatment and management of both types. While in T1DM, glucose regulation is remarkably effective in avoiding diabetic complications, clinical trials in T2DM are inconsistent. I review the clinical trial results with more sound statistical considerations in an attempt to resolve the inconsistencies and come out with a robust inference.
- As the most important component of the work, I propose a novel model of glucose regulation compatible with the emerging alternative view of T2DM that successfully resolves majority of anomalies haunting the field. This is accompanied by a number of testable predictions and suggested lines of further work needed to bring about a major conceptual revolution in the field.
- Ultimately I examine the possibility of a conceptual revolution or paradigm shift in the field in the light of the Kuhn's description of scientific revolution.

This line of work is at substantial deviation from the classical view of diabetes that has centred around glucose and insulin. However, insulin has a wide variety of functions apart from its role in glucose dynamics. A look at the functional evolution of insulin and insulin like molecules is therefore essential to begin with.

If we look collectively at the varied roles of insulin family of molecules, it looks apparently confusing and random. But a careful look reveals that they are all related to growth, reproduction and longevity. The three phenomena are components of what is called life history strategy in evolution. The life history theory (Roff 1993; Giovanetti 2012; Hawkes, O'Connell, and Jones 2003; Wit et al. 2013) depicts that a trade-off between growth rate and longevity or reproductive effort and longevity is inevitable and the widely different life histories in the animal world evolved from contextual optimization between these. The different roles of insulin suggest that this family of molecules primarily evolved for fine tuning of life history



strategies. Since different species evolved in different contexts, their optima were different. Also within a species, individual optima may also depend on the environment and developmental history of an individual. Therefore there is flexibility and development and environment dependent fine tuning of insulin action.

It is also possible that insulin action on glucose dynamics is also a part of this fine tuning. This is compatible with the Watve and Yajnik (2007) and Watve (2013) suggestion that insulin resistance is a behavioural fine tuning mechanisms. The evolved function of insulin is not to regulate plasma glucose but to allocate differential energy budgets to different organs and tissues thereby fine tuning the body's investment in growth, reproduction and longevity. It also means that plasma insulin level needs to be regulated not only for glucose dynamics but for any of the other functions of insulin as well. This needs to be taken into account in modelling glucose-insulin dynamics. This view of insulin action changes the perspective substantially and has implications for T2DM which I will explore in further chapters.



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CHAPTER 2

Cross species evaluation of relationship between insulin resistance, hyperglycemia and life span



2.1 Introduction:

In this chapter we attempt to understand the evolution of insulin function and insulin resistance in animal kingdom. Our interest in this was piqued by research on Mexican cavefish, where it was shown, that these fishes had evolved insulin resistance as means of prolonging their life span in response to harsh environmental condition. Mexican cavefish, *Astyanax mexicanus*, a classic example of evolution in response to local conditions, has recently attracted attention because it exhibits a condition like diabetes.

The teleost fish has two distinct morphs: surface fish, which have fully developed eyes and dwell in open streams and cave dwelling fish, which arose from surface fish ancestors that got locked in a cave environment and in due course of time lost their vision as well as pigmentation (Jeffery 2008; Yoshizawa 2015). The blind fish are also larger compared to surface dwellers (Jeffery 2008; Riddle *et al.* 2018). Although these two morphs look so different, they are interfertile and can produce viable offspring (Yoshizawa 2015). The cavefish have multiple populations residing in different caves and have achieved the blind phenotype independent of each other.

A loss of vision is accompanied by a series of behavioral changes related to feeding (Aspiras *et al.* 2015), mating (Plath *et al.* 2006), schooling (Kowalko *et al.* 2013) and loss of circadian rhythm (Aspiras *et al.* 2015). The cavefish were found to be resistant to starvation and lost only half as much weight compared to surface fish after two months of starvation. This phenomenon was attributed to much higher body fat and low metabolic rate in cavefish (Moran, Softley and Warrant 2014; Aspiras *et al.* 2015). With normal feeding, some of the cavefish showed hyperphagia and accumulated more fat and had fatty liver compared to surface fish.

Mutations in melanocortin 4 receptor (MC4R) were at least partly responsible for increased feeding, growth rate and starvation resistance in the cavefish (Aspiras *et al.* 2015). Mutation in the same region of MC4R gene is associated with severe obesity in humans as well although the condition is uncommon (Kahn, Hull and Utzschneider 2006). Compatible with these observations is the finding that the cavefish are perpetually hyperglycemic, a condition that we generally recognize as diabetes and associate with obesity (Riddle *et al.* 2018). The story so far is compatible with the classical thrift-obesity-diabetes theory (Neel 1962).



However, there are important twists and surprises in the story. We believe that in humans obesity causes insulin resistance, but in cavefishes, a P211L mutation in insulin receptor leads to insulin resistance and is at least partially responsible for the obese phenotype. When the mutant insulin receptor found in cavefish population was inserted in zebrafish, they also acquired the obese phenotype (Riddle *et al.* 2018). So here insulin resistance is primary, and

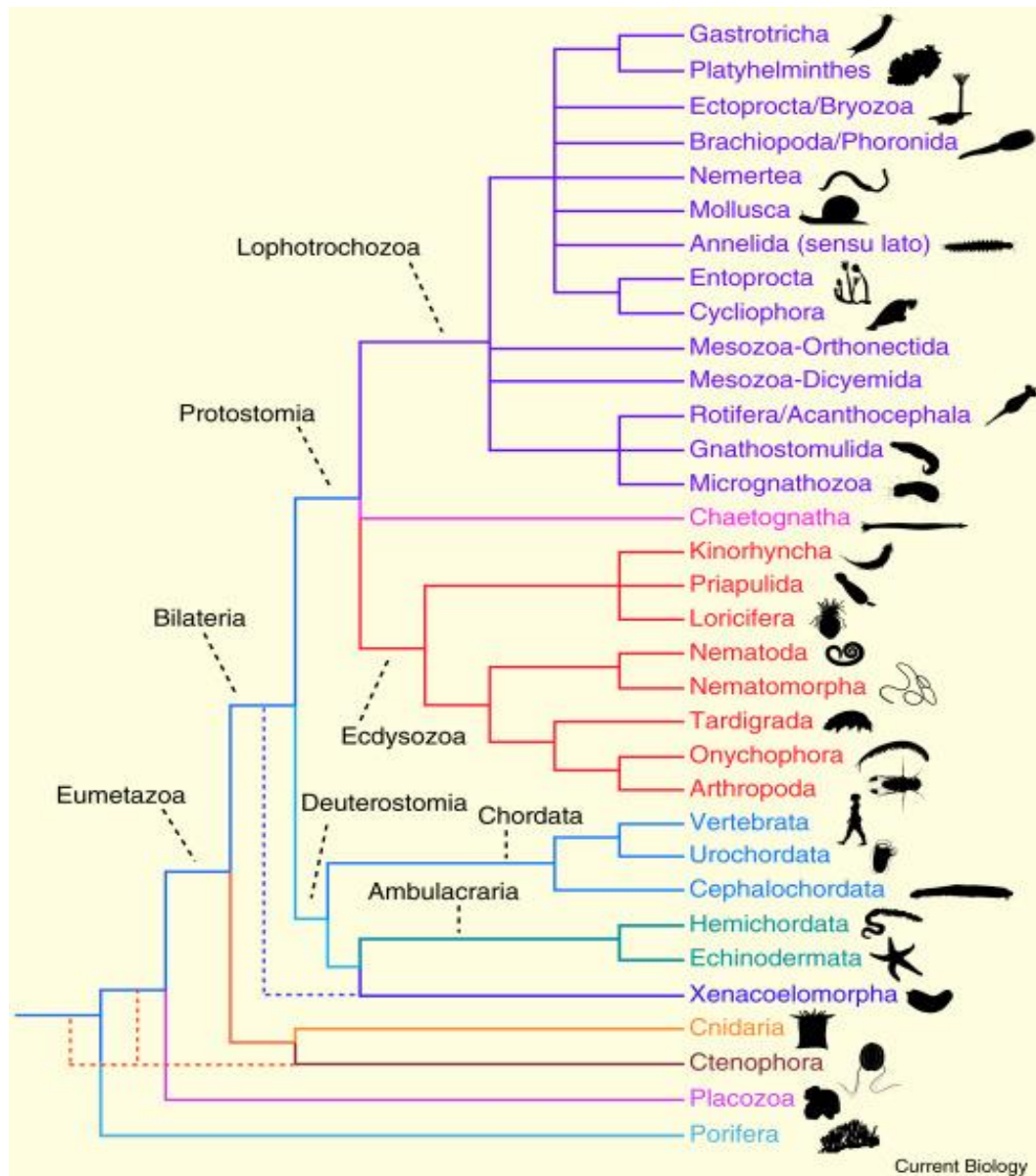


Figure 2.1: Phylogeny of animal kingdom. Adapted from Telford, Budd and Philippe, (Telford, Maximilian J., Graham E. Budd, and Hervé Philippe. "Phylogenomic insights into animal evolution." *Current Biology* 25.19 (2015): R876-R887.

obesity is the consequence. This observation is also similar to the result in mice that muscle-specific insulin receptor knockouts become obese (Brüning *et al.* 1998). This result is compatible with James Neel's original idea that a gene responsible for diabetic tendency leads



to obesity (Neel 1962), but not compatible with the notion that obesity induces insulin resistance. However insulin receptor mutations known in humans including the Donohue syndrome or the Rabson Mendenhall syndrome are non-obese and have post-prandial hyperglycemia but fasting hypoglycemia (Semple *et al.* 2011). In the pathophysiology of type 2 diabetes, the cause-effect relationships are often unclear. Different schools of thought appear to propagate different and often contradictory causal relationships. The thought that obesity leads to insulin resistance dominated over a few decades and multiple mechanisms for obesity induced insulin resistance were proposed (Kahn and Flier 2000; Kahn, Hull and Utzschneider 2006; Qatanani and Lazar 2007; Eckel *et al.* 2011; Odegaard and Chawla 2013; Ye 2013; Hardy, *et al.* 2012). Part of the evidence is based on correlations (Roden *et al.* 1996; Boden 1997; Kadowaki 2000; Perreault and Marette 2001; Xu *et al.* 2003; Yang *et al.* 2005) and even the correlations are weak (Vidwans and Watve 2017). The old view that higher insulin response leads to obesity (Neel 1962) is gaining grounds once again (Astley *et al.* 2018). Further, insulin resistance in the cavefish is not accompanied by increased levels of insulin meaning thereby that compensatory hyperinsulinemia does not inevitably follow insulin resistance (Riddle *et al.* 2018). Knocking out insulin receptors from muscle or fat tissue in mice also did not result in hyperinsulinemia (Brüning *et al.* 1998). In contrast insulin receptor mutants in humans (Donohue syndrome or the Rabson Mendenhall syndrome) are hyperinsulinemic and have fasting hypoglycemia (Semple *et al.* 2011). Thus, there is no agreement on the causal relations between obesity, insulin resistance and hyperinsulinemia and causalities can be different in different species.

Chronic hyperglycemia in humans is believed to lead to a variety of complications which often take a lethal turn. However, the cavefish remain healthy and long-lived with their hyperglycemia. One of the main mechanisms of hyperglycemia induced complications is believed to be increased glycation of proteins (Wautier and Guillausseau 1998; Stirban, Gawlowski and Roden 2014). Among the independently evolved lines of fish, some appear to be relatively resistant to glycation, but others are not, and still, all of them are long-lived without any signs of diabetic complications (Riddle *et al.* 2018). It is likely therefore that either hyperglycemia is not the real cause of diabetic complications or it is possible to evolve multiple ways to avoid pathological consequences of hyperglycemia, if any. Compatible with this is the failure of clinical trials targeting strict glycemic control to arrest diabetic complications (Turner 1998; King, Peacock and Donnelly 1999; Group *et al.* 2008; Ismail-Beigi *et al.* 2010).



Therefore, whether hyperglycemia is the predominant causal factor behind the pathological consequences of diabetes needs to be re-examined.

2.2 Cross species variations in insulin response, insulin resistance, hyperglycemia and lifespan

Cross species comparison is an important tool in evolutionary medicine, but so far data on insulin resistance in non-human species is uncommon. We tried to explore the evolution of insulin function across the known phylogeny of animal kingdom (Figure 2.1). Most of the research available for insulin resistance exist for vertebrates, with few exceptions of *C. elegans* and *D. melanogaster* as non-vertebrates. Although fragmentary, it is possible to put together data on insulin resistance in cavefishes, black and grizzly bears (*Ursus thibetanus*, *Ursus americanus*, *Ursus arctos*) (Kamine *et al.* 2012; McCain, Ramsay and Kirk 2013), horses (*Equus ferus*) (Rapson *et al.* 2018), chimpanzees (*Pan troglodytes schweinfurthii*) (Emery Thompson *et al.* 2009), bonnet macaques (*Macaca radiata*) (Kaufman *et al.* 2005) and dolphins (*Tursiops truncatus*) (Venn-Watson *et al.* 2013) to see the commonalities and differences. Insulin signaling is involved in secondary sexual characters in many species as wide as beetles (Emlen *et al.*, 2006; Okada *et al.*, 2019) and deer (Bartos *et al.*, 2012). Overgrowth of a certain organ is achieved by making the specific tissue more insulin sensitive than the rest of the body. Hibernating animals exhibit a physiology which allows them to survive extreme temperatures by manipulating their metabolism. American and Japanese black bear revealed a seasonal variation in insulin sensitivity during summer/spring active period and fall/winter hibernating period (Kamine *et al.* 2012; McCain, Ramsay and Kirk 2013). Grizzly bears undergo a similar annual metabolic cycle: in active period they gain weight up to 4kg per day and lose as much as 50% of their body weight during for the 5-7 months of hibernation (Nelson *et al.* 2003; Folk *et al.* 1974; Erlenbach *et al.* 2014; Rigano *et al.* 2017). Females with higher fat content have higher fecundity and may produce more viable offspring (Atkinson and Ramsay 1995; Robbins *et al.* 2012). This seasonal fat deposition was seen to be a result of a changed insulin sensitivity in black bear as well as brown bear (Kamine *et al.* 2012; McCain, Ramsay and Kirk 2013; Rigano *et al.* 2017). In summer, accompanying fat accumulation, the bears were more insulin sensitive. In contrast insulin concentration was 2.5 times higher in hibernating grizzly bear, although they were euglycemic (Rigano *et al.* 2017). Interestingly when the adipocytes from



hibernating grizzly bear were treated with serum from summer active grizzly bear, insulin sensitivity was partially gained by their adipocytes (Rigano *et al.* 2017). Contrary to humans, fat deposition appears to be associated with insulin sensitivity and fat loss with insulin resistance in bears.

Dolphins have also been shown to exhibit insulin resistance, but this is a response to high protein diet (Venn-Watson *et al.* 2013). Free ranging dolphins have lower plasma glucose and insulin levels compared to captive population, but both show increased insulin resistance with high protein diet (Venn-Watson *et al.* 2013). This observation contrasts the rodent models of high fat feeding to induce insulin resistance (Oakes *et al.* 1997). Insulin sensitivity in horses varies in different breeds (Bamford *et al.* 2014) and decreases with age in mares, but there was no difference in mares fed with high sugar or high fiber diet (Rapson *et al.* 2018). In Chimpanzees and Bonnet macaques, insulin resistance is observed in a fraction of the population normally in the absence of any obesogenic intervention (Kaufman *et al.* 2005; Emery Thompson *et al.* 2009). Further in chimpanzees, the low social rank individuals were more insulin resistant than the high ranking individuals (Emery Thompson *et al.* 2009) although the high ranking individuals have greater access to food. The association of insulin resistance with food intake appears to be negative here.

Studies on different species of vertebrates seem to suggest that the relationships between food intake control, fat accumulation, insulin resistance, insulin production, hyperglycemia and its pathological effects have evolved differently in different species. It is not necessary that insulin resistance in all species follow obesity. Simple biochemical mechanistic are not adequate to explain the variable relationship observed between fat accumulation, insulin resistance and hyperglycemia. Most evolutionary hypotheses for human type 2 diabetes stop at explaining obesity and assume that all other effects follow inevitably from obesity. This is not sufficient for an evolutionary hypothesis. If obesity induces insulin resistance in humans but not in all species, it is necessary to explain why this relationship evolved specifically in some species including humans. So far, the obesity centered evolutionary hypotheses about type 2 diabetes are far from satisfying this condition.

A more generalizable inference can be that insulin mainly decides differential investment of the body in different organs, growth or activities. This way it helps fine tune different behavioral strategies adopted by the organism in a given ecological and social environmental



context. This perspective of insulin function contradicts the classical medical perspective of insulin as a glucose regulation molecule and supports the life history and behavioral optimization mediator molecule of Watve and Yajnik (2007) and Watve (2013). The insulin-glucose dynamics needs to be interpreted in this context.



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CHAPTER 3

Collective analysis of clinical trials towards
intensive glucose control treatment



3.1 Introduction:

“Absolute proof that good (glycemic) control can retard or prevent the development of complications has not yet been obtained, but the assumption is accepted by most diabetologists and indeed is almost an article of faith in the current approach to achieve the best possible diabetic control” Geoffrey Gill (1991) (Williams J C, 1991).

Diabetes was initially identified by the presence of sugar in the urine, dating back to ancient times. Subsequently, elevated blood glucose levels became recognized as a hallmark of diabetes. Insulin, a pivotal discovery made approximately a century ago, has been the focal point in our understanding of diabetes. However, contemporary research has unveiled the involvement of numerous molecular, cellular, and neuronal signals, surpassing a hundred, that are known to be altered in diabetes (Watve, 2013).

Network models attempting to integrate these diverse signals have raised doubts about the central and causal role traditionally attributed to glucose and insulin in the pathophysiology of diabetes (Kulkarni et al., 2017). Nonetheless, prevailing clinical practices and perspectives continue to revolve around glucose and insulin. Over the course of several decades leading up to the mid-20th century, the differentiation between type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) gradually became clearer (Colman et al., 1999; Dana, 1954; Harris, 1988; Himsworth, 2013; Null et al., 1973; Yki-Järvinen et al., 1986).

Insulin has proven highly successful not only in regulating glucose but also in mitigating complications associated with T1DM (Fullerton et al., 2014; Nathan, 2014; Nathan et al., 2009). Due to the perception that T1DM and T2DM differ primarily in the underlying cause of glucose dysregulation, and that the downstream pathophysiology resulting from chronic hyperglycemia is nearly identical in both types, the effectiveness of glucose regulation observed in T1DM was anticipated to extend to T2DM as well.

However, early clinical trials investigating glucose regulation in type 2 diabetes demonstrated that it was not as effective in mitigating diabetic complications as observed in type 1 diabetes (Huang et al., 2001; Skyler JS et al., 2005). The efficacy of glucose regulation in type 2 diabetes remains a subject of debate (Boussageon et al., 2017). While the ultimate goal of treatments for type 2 diabetes is the prevention of complications, the immediate surrogate goal is often believed to be the normalization of glucose levels. This approach is based on two common assumptions: (i) increased glucose levels are causally linked to diabetic



complications, and (ii) normalizing glucose can significantly reduce the occurrence of complications.

The first significant challenge to these assumptions arose from the findings of the University Group Diabetes Program (UGDP), in which glucose normalization failed to decrease complications and mortality; instead, it led to an increase in some treatment arms (Miller et al., 1976). UGDP faced criticism and controversy, prompting subsequent decades to witness numerous clinical trials employing different study designs, sample sizes, drugs, and outcome measures. While some trials reported significant reductions in specific adverse outcomes, others perplexingly found increased cardiovascular and all-cause mortality in the intensively controlled group (NICE Investigators, 2009; Ismail-Beigi et al., 2010).

To address the conflicting results, particularly regarding macrovascular outcomes, a collaboration was established among four major trials: UKPDS, ACCORD, ADVANCE, and VADT. A meta-analysis of these trials concluded that intensive glucose regulation achieved a "modest decrease" in macrovascular events (Turnbull et al., 2009).

However, several issues remain unaddressed regarding the scientific rigor of trial designs, statistical treatments employed, and the inferences drawn from the results. In this thesis, we aim to discuss the potential pitfalls and biases present in these studies, explore alternative interpretations of the findings, and evaluate the scientific validity of the current assumptions underlying the treatment of type 2 diabetes. We undertake a comprehensive analysis of large-scale institutional, multicentre, multi-drug trials on glucose normalization, with long-term follow-up, expanding upon the scope of the meta-analysis conducted by Turnbull et al. (2009). Through this analysis, we re-examine the design, statistical analysis, inference-related issues, and clinical implications of these trials.

The need for scepticism regarding published randomized clinical trials (RCTs) and the critical examination of their validity has become increasingly important over the past decade or two (Chow et al., 2021; Heneghan et al., 2017; Herrera-Perez et al., 2019; Ioannidis, 2016). Efforts have been made to introduce reforms and enhance the rigor of RCTs. One such measure is the mandatory registration of RCTs prior to participant recruitment. Mandatory registration aims to address issues such as failure to report, partial reporting, suppression of inconvenient results, manipulation of study norms, and concerns regarding data transparency (James et al., 2015; Ramsberg & Platt, 2018; Thabane et al., 2015; Tse et al., 2009; Zarin & Keselman, 2007).



Despite the increasing awareness and ongoing efforts to improve the design of clinical trials, many challenges still persist. Moreover, a significant number of clinical trials investigating glucose normalization in type 2 diabetes (T2DM) were initiated prior to the recent advancements in refining clinical trial designs. Therefore, it is crucial to specifically examine the potential biases present in glucose regulation trials for T2DM and evaluate their impact on the robustness of inferences and clinical applicability.

3.2 Methodology:

3.2.1 Selection of studies and inclusion/exclusion criteria:

This article focuses on both qualitative and quantitative issues. For the quantitative analysis in this study, we included institutional clinical trials that met the following criteria: a minimum of 100 individuals per group, a minimum follow-up period of 3 years, and reporting rate ratio (RR), odds ratio (OR), or hazards ratio (HR) (or data sufficient to calculate these indices) for multiple macro and microvascular events and mortality. We excluded trials that reported only surrogate outcomes and trials conducted by pharmaceutical sector companies during drug development. The selected trials based on these inclusion-exclusion criteria are the University Group Diabetes Program (UGDP), the UK Prospective Diabetes Study (UKPDS), the Veterans Affairs Diabetes Trial (VADT), the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE), the Action to Control Cardiovascular Risk in Diabetes (ACCORD), and the Diabetes Prevention Program (DPP).

For analysis, we included all comparisons between a treatment or intensive treatment group and a control group without the specified treatment regime, with or without blinding/placebo. We excluded comparisons between different drugs. Additionally, we excluded trial arms with specific drugs that were discontinued, such as tolbutamide in UGDP or troglitazone in DPP. As the necessary data for the intended analysis were available in the published papers, there was no need to access raw data during the study.

The endpoints/outcomes included in the analysis were those that had at least 10 events in at least one of the compared groups. We considered single endpoints and aggregated outcomes reported in tables, figures, and text. It is unclear why some results were reported in tables while others were only mentioned in the text, but we deemed it necessary to evaluate all reported information. Subgroup analyses and associative analyses that attempt to relate glycemic status



to the incidence of complications while ignoring the allocation to trial regimens, such as in Hayes et al. (Hayes et al., 2013) or Stratton (Stratton, 2000).

Given the variations in trial designs, patient characteristics, and outcome definitions across studies, and considering that a meta-analysis approach has been used previously, we do not employ a meta-analysis approach in this study. Instead, we critically examine the reported results collectively, focusing on unresolved or inadequately addressed issues from earlier literature.

3.2.1 Simulation model

A simulation model was developed in which an appropriate randomization is done to study the effect of sample size, number of outcomes, incidence, and interdependence of the outcomes on the significance of the difference observed in treated vs control group. A baseline model assuming independence: We generate two randomized groups of participants with the same size n . For each outcome there is an incidence i (always <1) which varies within a range. The treatment effect, if any, is t , ranging from 0 to 1. No treatment effect is represented by $t=1$ and for 100% effect $t=0$. For modelling chance alone we keep $t = 1$.

For every outcome we take a random i within a realistic range. We generate incidence of the outcome within the two groups. For everyone out of the n individuals, we generate a random number (RND) bet 0 and 1, if $RND < i$ the outcome is seen, if $> i$ it is not seen. This is repeated independently this for all n individuals. The individuals showing the outcome are counted. For the treated group the same is repeated using $RND < i.t$. A chi square test is performed to see whether the difference in the treated and control group is significant, simultaneously the odds ratio (OR) is calculated.

For the next outcome a new random i is generated and the process repeated for each of the o number of outcomes. At the end, the frequency distribution of total ORs is plotted, indicating those that are significant. A detailed description and the code is provided in Appendix I.

3.3 Observations:

3.3.1 Inadequacies of the studies:

We observe that the main issues unaddressed or inadequately addressed across the selected trials are as follows.



1. Analysis of repetitions and interdependencies among outcomes in clinical trials comparing intensive and conventional control groups

Summing up over the 6 clinical trials (UGDP, UKPDS, VADT, ACCORD, ADVANCE and DPP) out of the total 341 outcomes compared between intensive and conventional control groups, in 38 pairs the frequency of adverse outcomes is individually significantly lower than the control and in 26 it is individually significantly higher.

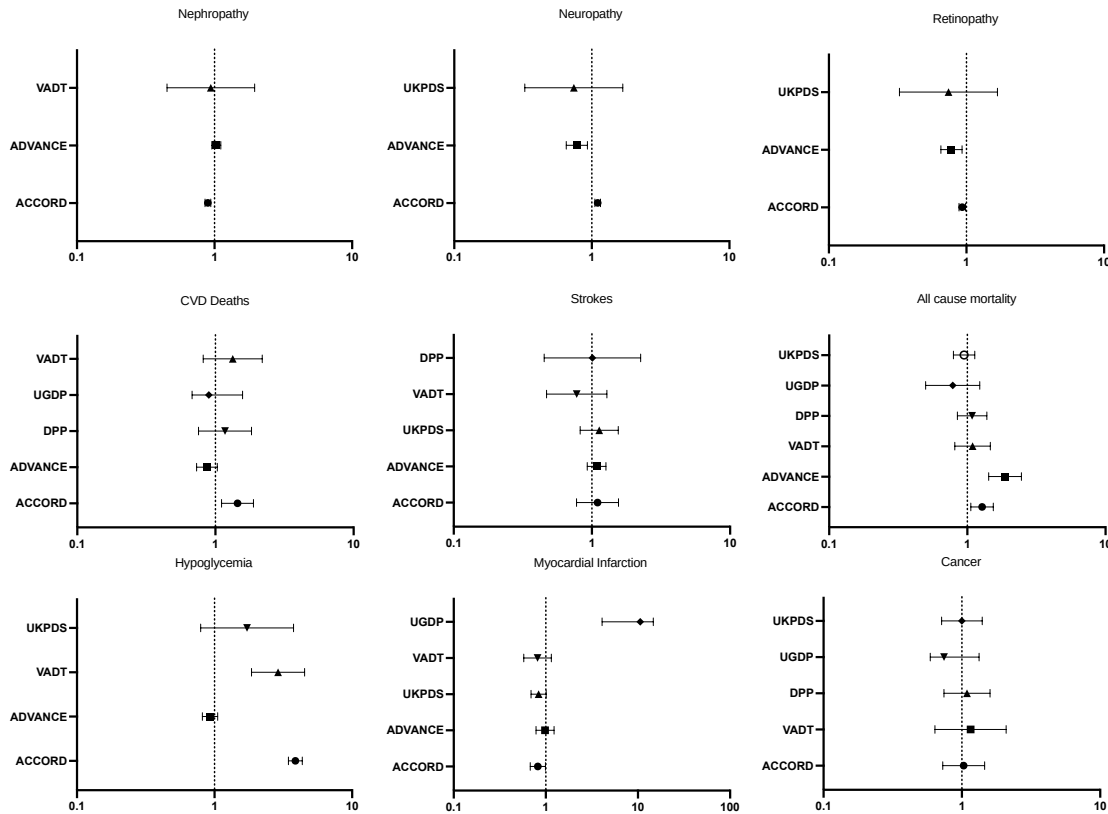


Figure 3.1: Comparison of effect of intensive glucose lowering and conventional glucose lowering in clinical trials.

However, there are repetitions and interdependencies among these outcomes. For example, all-cause mortality includes cardiovascular mortality, so if the latter is significantly different, the chances of the former being significant increase. By eliminating such obvious duplications, we find that 17 adverse outcomes show a significant reduction, while 13 show a significant increase when considering all 6 trials. By this simple summation, it cannot be concluded that the beneficial outcomes are significantly greater than the harmful outcomes (Figure 3.1). If soft, subjective, and surrogate endpoints are excluded, there is no difference in the number of beneficial and harmful results.



2. Appropriate use of significance level when multiple comparisons are made: When multiple statistical comparisons are made, it is possible that some of them turn out to be “significant” by chance alone. This is a well-known statistical problem and the suggested solution is to decrease the significance level in proportion to the number of statistical tests used (Narum, 2006; Rice et al., 2008). None of the clinical trials have used any of the suggested corrections for significance level used. Turnbull et al (Turnbull et al., 2009) recognized this problem but did not attempt any solution. The total number of outcome measures reported in these trials is large. If the Bonferroni principle or any other correction for significance level is applied, none of the effects of treatment turn out to be significant. Since the Bonferroni correction is too conservative, we look at how many outcomes are expected to be significant at 0.05 level by chance alone and whether the number of individually significant results observed in the trials are significantly greater than the expected.

However, when multiple statistical tests are conducted, how many will turn out to be individually significant by chance alone is not an easy question to answer when the different outcomes are not independent. There are two types of dependencies in the glucose normalization trial data. One is that of repetition in aggregate end points as mentioned above. The other is that of having common pathways/mechanisms behind many outcomes. If the common pathway happens to differ between the two groups by chance, a number of outcomes may appear to be significant together. This problem has not been addressed in the statistical treatment of clinical trials with multiple outcome measures so far.

We address this problem using simulations (see Appendix 1 for details of the simulation model). The simulations generate the control and treated group and the outcome in each individual in the group is generated by appropriate randomization. The ORs resulting from these simulations are studied. In the baseline simulations where treatment effect is assumed to be zero and each outcome is assumed to be independent and have the same parameters, the ORs are distributed with a mode around unity. Being ratios, the distribution is positively skewed as expected and the ORs at both the tail ends are individually significant. However, in reality the incidence of every outcome is substantially different, for example, in the ADVANCE trial visual impairment was seen in 54 % patients but dementia in only 0.9%. When differences in incidences are incorporated in the simulations, not only the tails but ORs with intermediate departure



from unity on either side are more likely to be significant. This is because at low incidence, a greater departure from unity is possible by chance alone, but significance is difficult owing to the smaller number of cases. On the other hand with larger incidence, moderate departures from unity also turn out to be individually significant. This pattern matches with the clinical trials data (Figure 3.2).

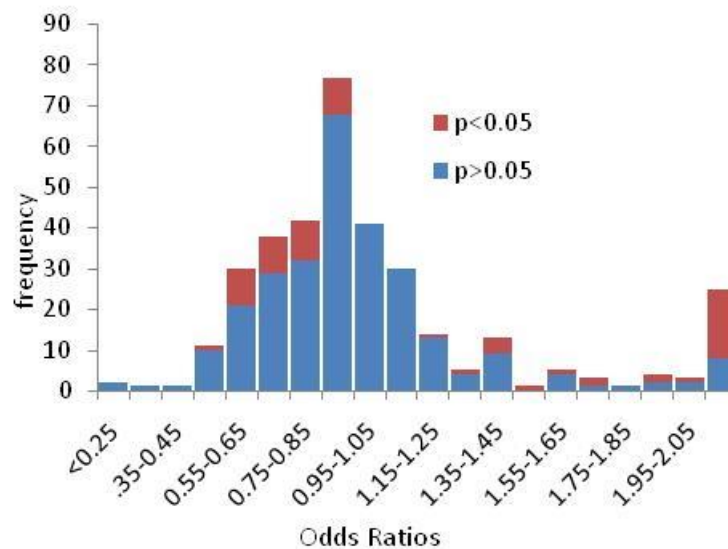


Figure 3.2: The frequency distribution of odds ratios for all outcomes reported in the six clinical trials. The mode and median have shifted slightly to the left from unity, but this difference cannot be said to be statistically significant. Individually significant statistical deviations are on both sides. The geometric mean of the population is close to one (1.01) indicating near symmetry in magnitude.

The simulations can incorporate interdependence in different ways. By the classical assumption hyperglycemia is the common trigger behind all pathophysiology of diabetic complications. If this assumption is true, all outcomes should be interdependent. Alternatively it is also possible that some clusters of outcomes are interdependent but independent of other clusters. This assumption is relevant to T2DM since the factors involved in microvascular and macrovascular complications can be different. Further in some tissues capillary density appears to reduce where as there is hyper-angiogenesis in some other complications. Simulations show that by either modes of dependence, in a large proportion of simulation runs the proportion of outcomes individually significant can be substantially greater than the expected 5%.



Therefore having greater than 5% outcomes individually significant cannot be taken to reflect the effect of treatment. Since we do not have empirical estimates of the extent of interdependence, we cannot state how many outcomes can turn out to be individually significant by chance alone.

A possible solution to the problem is that instead of depending upon individual level significance, we look at the distribution of all ORs. If there is a favourable treatment effect on the common mechanism itself, as is assumed by the classical theory of T2DM, then the entire distribution should shift to the left. The significance of this shift can be judged by multiple simulation runs. We observe that when pooled over all the six trials, the distribution of ORs has a mode and median shifted to the left by 10 % and 7.3 % respectively (Figure 3.2). By this indication, intensive glucose control may be said to have a benefit of 7 to 10 % which is compatible with the estimate of 9 % obtained by Turnbull et al (Turnbull et al., 2009). This contrasts with the distribution of ORs in T1DM where almost the entire distribution of ORs lies to the left of (DCCT group 1987; Fullerton et al., 2014; Nathan, 2014). Similarly for exercise intervention for T2DM across studies, almost all ORs are less than 1 (Sluik et al., 2012). The distribution of ORs in the T2DM trials has only a marginal leftward shift. Whether this shift is significant or not is questionable. In simulations comparing 350 outcomes, comparable to the pooled data of all 6 trials, 10% or greater shift in the mode and the median was observed in about 16 % of simulation runs (see Supplementary information). Therefore the shift observed in the pooled 6 trial data may not be considered significant.

If we assume that some clusters of outcomes have some common underlying pathway and are therefore interdependent, it is seen that a greater than expected proportion of outcomes can turn out to be individually significant by chance alone in a substantial proportion of the simulation runs. However, they may be symmetrically or asymmetrically distributed towards the two tails. Two or more simulation runs can result in significant heterogeneity among them although they are run at the same parameters.

Since we do not have empirical data on the extent of interdependence, simulations cannot be used to make quantitative predictions. But they conclusively show that if the assumption of independence of chance acting on every outcome is violated, the number of false positive significance increases substantially. Unless this effect is accounted for, we cannot conclude that the reduction in some of the outcome measures claimed in the



treatment groups is indeed significant and not a false positive. It is equally likely that the significant rise in mortality seen in certain trials such as tolbutamide arm in UGDP, the metformin plus sulfonylurea group in UKPDS or in the rosiglitazone trial (RECORD) (Home et al., 2009) also may be chance alone. If this is true, other inferences such as glucose normalizing treatment being more effective for microvascular outcomes than for macrovascular ones is equally unfounded. Further the observed heterogeneity between trials is also possible by chance alone.

In short since we know that certain common mechanisms are involved in the pathophysiology of diabetic complications, we should expect a large number of false positive results as well as heterogeneity between trials. On this background, the observed individual outcomes of the trials are marginal and therefore any inferences cannot be confidently made. The shift in the mean, mode or median of the distribution is also not significant and therefore the hypothesis that hyperglycemia is the common pathophysiological mechanism of all complications and anti-hyperglycemia treatment can arrest complications is not supported.

3. Placebo effects: Having a double blinded placebo controlled design is a standard norm for almost all clinical trials, unless a specific context makes blinding impossible. While ACCORD, ADVANCE, VADT, UGDP and DPP have some kind of blinding or placebo control, whereas UKPDS is an open label trial. Plotting the distribution of ORs in the trials with and without placebo reveals that the shift in mean and median to the left is observed for UKPDS alone which does not have a placebo control (Figure 3.3). The distribution of ORs in the pooled data of trials with placebo have a mode at 1 and do not show a left shifted distribution of ORs. This raises the possibility that the apparent benefit of treatment claimed in UKPDS may only be a placebo effect. DPP allows comparison of a placebo group with a no medication group within a single trial. There the difference is not statistically significant but is of a comparable order (Lee et al., 2021). Some other studies have also demonstrated detectable placebo effects in diabetes treatment (de Wit et al., 2016; Sievenpiper et al., 2007).

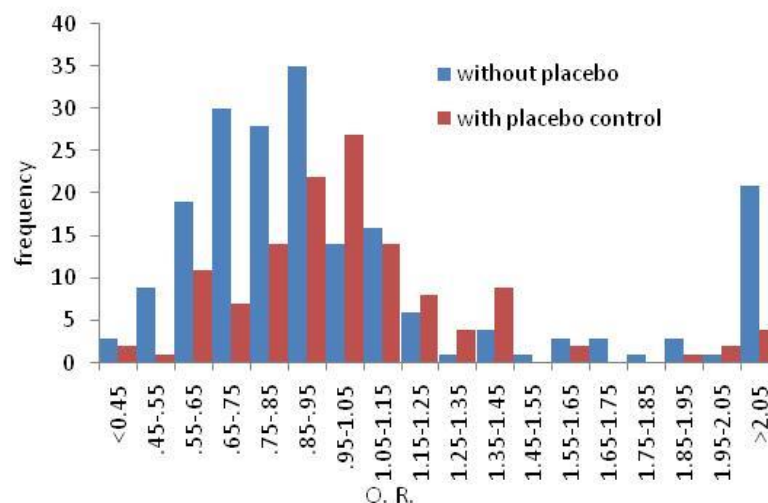


Figure 3.3: The distribution of ORs in trials with and without placebo control.

There is a leftward shift in the distribution without placebo control. The marginal leftward shift seen in the total (figure 3.1) can therefore be suspected to be a placebo effect.

In clinical trials with surrogate markers, placebo effect can potentially operate at two distinct levels. One is in receiving the drug versus the blank. The second possible level of a placebo effect i.e. knowing that my blood sugar is under better control can potentially have positive psychosomatic effects. If a reduction in the frequency of complications or mortality is observed, it may be potentially explained by the second level placebo effect. So far, to the best of our knowledge, no trial has attempted to address the potential second level placebo. Unless this possibility is seriously considered, tested using appropriate controls and rejected, the marginal beneficial effects observed cannot be confidently claimed to be due to better glucose regulation.

4. Even if we assume that the marginal benefits observed in some of the outcomes are not by chance, it does not show that they are a result of glucose regulation. Across as well as within trials, the difference in HbA1c achieved and the relative success in preventing complications do not always correlate well. Within UKPDS, the sulfonylurea and insulin arms had HbA1c difference of 0.9 but a significant difference was seen only in the soft endpoint of need for retinal photocoagulation (Robert Turner, 1998). In the post-trial follow up the difference in HbA1c had vanished but a greater benefit of intensive control was apparent (Holman et al., 2008). Unlike UKPDS, a post-trial long term follow up in VADT and DPP did not show any long term benefits (Lee et al., 2021;



Reaven et al., 2019) demonstrating the lack of consistency across trials. In the overweight patients group of UKPDS, the advantage obtained by metformin over sulphonylurea and insulin was not explicable by better glycemic control (R. Turner et al., 1998). Across trials ACCORD and ADVANCE achieved the largest HbA1c difference, but they do not exhibit the greatest success in arresting complications.

Other studies show an association of glycemic status with the risk of complications, but these studies cannot be used to make a causal inference (Stratton, 2000). It is possible that for individuals with a more serious underlying pathology, the chances of complications are higher as well as glycemic control is more difficult. This results in a correlation but does not imply that reducing glycemia would result in risk reduction.

Many of the drugs used in treatment of T2DM have multiple effects in the body independent of glucose regulation. Insulin is known to have multiple functions in the body ranging from amino acid metabolism, cell division to cognitive function and ovulation regulation (Shemesh et al., 2012; Strachan, 2005). Metformin is shown to affect endothelial function directly (Diamanti-Kandarakis et al., 2005; Heidari et al., 2019). The beneficial effects of SGLT2 inhibitors are seen in diabetic and non-diabetics alike (DeFronzo et al., 2019; Ferrannini & DeFronzo, 2015). Therefore even if any beneficial effects of any of the drugs in diabetes treatment are seen, it cannot be stated that the benefits are because of improved glucose regulation. Even in T1DM whether the observed efficacy of insulin treatment is because of glucose regulation or because of the multiple other functions of insulin has not been clearly addressed and resolved.

5. The magnitude of difference: Although the number of outcomes with individually significant beneficial effects of treatment are marginally greater than the number of outcomes with increased frequency, the frequency of harmful effects is substantially greater. While the individually significant reductions in ORs range between 0.5 and 0.92, the increase ranges between 1.2 and 13.1. The harmful effects of intensive treatment not only include major hypoglycaemic events and other drug related symptoms, but also cardiovascular mortality, cancer and all-cause mortality in different trials. By the properties of ratios, the distribution of OR is bound to be positively skewed. The geometric mean of all ORs is close to one (1.01) indicating that the net odds ratios do not deviate from unity. Therefore it cannot be stated that the benefits, if any, of intensive glucose regulation outweigh its harmful effects.



Even if, for the time being, we ignore the harmful effects and focus on the beneficial ones alone and assume that the claimed significance was real, whether the benefits are clinically meaningful is a crucial question. Although in public health literature, the emphasis is on ratio based indices such as OR, RR and HR, common people seem to go by absolute risk reduction (ARR). In an experiment, respondents including the ones having undergone training in public health statistics were observed to judge their own risk by probability difference rather than by probability ratio (Patil et al., 2021). In all glucose regulation trials for T2DM, the ARR is too small to be clinically meaningful. For example, in ADVANCE trial over a 5 year follow up, the incidence of combined major microvascular and macrovascular events was 20% in the control group and 18.1% in the treatment group. Although by ratio based indices this is about 10% reduction, by difference based indices it is only 1.9%. The number needed to treat (NNT) for preventing diabetic complications in any of the trials is 20 and above even after ignoring the increased frequency of some adverse events. Since different trials have different follow up periods, and the effect appears to be more or less linear consistently over time, we can express it as NTNT, i.e. number-time (person-years) of treatment needed for preventing one complication (Laupacis et al., 1988). NTNT is fairly consistently distributed across trials and averages to about 250. That is if 25 diabetics are treated targeting glucose regulation for 10 years, one complication in one of the 25 patients may be prevented. This is quite a low success rate. Whether at this success rate it is worth undergoing treatment for glucose control, that has a considerable frequency of adverse events is a subjective decision which should be left to the patient after having informed about NTNT in a simple language. Failure to inform the patient about the efficacy of treatment seen in clinical trials potentially amounts to a violation of human rights.

Apart from the trials included here, there are many more that converge on the same inference. These trials did not fit into our inclusion exclusion criteria but their inferences converge with our analysis. The NICE-sugar study revealed that under critically ill patients tight sugar control resulted in higher mortality (NICE Investigators, 2009). Huang et al meta-analysed 5 glucose regulation trials for their effect on cardiovascular disease (Huang et al., 2001). Apart from UGDP and UKPDS they included VACS DM, Kumamoto and DIGAMI trials and the meta-analysis yielded no effect of glucose lowering on cardiovascular disease.



In short, owing to multiple flaws in the design, statistical analysis and inferential logic in the clinical trials of glucose regulation in T2DM, at present we cannot state with scientific rigor that glucose regulation has any clinical benefit in T2DM, in contrast with T1DM.

This brings into question the classical assumption that T1DM and T2DM differ only in what goes wrong in glucose regulation and all downstream effects of hyperglycemia are similar. It is possible that the two are fundamentally different and converge only on one of the symptoms that is hyperglycemia. Hyperglycemia may not be central to the pathophysiology of the two in a similar way. Therefore the target of treatment of the two needs to be different.

3.3.2 Evidence for confirmation bias:

Although the difference between the response of T1DM and T2DM to treatment targeting glucose regulation is evident for quite a few decades, there is a deep rooted reluctance to accept the evidence and refute glucose lowering as the main line of treatment in T2DM. We argue that this is because of confirmation bias which is evident in multiple ways in the analysis and presentation of data in the publications resulting from the clinical trials.

a. Reporting positive and negative outcomes with different statistical treatment:

At times, for reporting two different results in the same paper, two different reporting formats are used without justifying the difference. Quoting verbatim from the reported UKPDS results (UK Prospective Diabetes Study (UKPDS) Group, 1998),

“Compared with the conventional group, the risk in the intensive group was 12% lower (95% CI 1–21, $p=0.029$) for any diabetes-related endpoint; 10% lower (–11 to 27, $p=0.34$) for any diabetes-related death; and 6% lower (–10 to 20, $p=0.44$) for all-cause mortality.”

In the following paragraph, the risk of major hypoglycemic events is reported as,

“The rates of major hypoglycaemic episodes per year were 0.7% with conventional treatment, 1.0% with chlorpropamide, 1.4% with glibenclamide, and 1.8% with insulin.”

It is strange that the risk reduction is given as percent reduction, but the incidence of hypoglycemic events reported as absolute frequencies. If the risk of hypoglycemic events is also expressed as percentage, with chlorpropamide it is 43% higher, with glibenclamide it is 100% higher and with insulin it is 170 % higher. Effectively the increase in risk of major hypoglycaemic event is up to an order of magnitude greater



than the maximum reduction seen in any of the complications in the trial. However, since the reporting format is different, a common reader is more likely to attach greater importance to percentage figures and attach smaller importance to hypoglycaemic events since the numbers reported are fractional. The tradition of reporting favourable and unfavourable results using different statistical expressions is continued in many other clinical trials of T2DM.

Another way of differentially reporting favourable and unfavourable results is including them in the figures or tables versus making a passing mention in the text. In UKPDS as well as in DPP many outcomes are mentioned in the text but not included in the tables/figures. The text sometimes has incomplete information to calculate OR, confidence intervals or statistical significance. Interestingly not a single outcome reported in the text shows significant favourable effect of treatment. There is no justification offered why these were not included in the tables. In DPP, for example, medication related gastrointestinal symptoms are mentioned in the text but not included in the tables. Participants that were unable to continue metformin due to adverse reaction are mentioned in the text, but these adverse events do not seem to be included in the analysis (White et al., 2022).

- b. Cherry picking: The UKPDS results (UKPDS group 1998a) say that the reduction in all diabetes related end points was 12% and statistically significant. However, this does not include major hypoglycemic events. If major hypoglycemic events are pooled with other adverse events, no different between control and treated group remains with respect to all outcomes. The primary reports of UKPDS enlist a large number of single point and aggregate outcome measures, but the post-trial follow up reports only seven aggregate outcomes. There is no way to know whether only the ones showing favourable results were selectively reported.
- c. Changing targets: The UKPDS study first decided to consider at least 40% reduction in mortality or morbidity in the intensive treatment group in order to be clinically significant. This benchmark was reduced to 15 % subsequently. But UKPDS has been criticised for not maintaining even the lowered standard and later declaring a 12% reduction as significant (Ewart et al., 2001).
- d. Recognizing but failing to correct flaws: The meta-analysis of 4 trials recognizes the flaw that the necessary adjustment of significance level for multiple statistical comparisons has not been made, but does not try to apply any correction for it (Turnbull



et al., 2009). The published reports of individual trials do not even acknowledge this serious flaw, which is quite well known in the field of statistics.

- e. Manipulating readers' perspective: Most readers stop after reading the title and abstract. Therefore selecting what to write in the title and abstract is an effective way to manipulate readers' perspective. For example the meta-analysis by Turnbull et al acknowledges that since they have not made correction for multiple comparisons, their analysis should be treated as tentative and exploratory, this admission does not figure in the abstract and therefore most readers after reading only the abstract are likely to take their results as robust and conclusive.

3.4 Conclusions

The most parsimonious explanation of all the reporting anomalies together is that it comes out of inadvertent or intentional confirmation bias and the burden of history. Since the substantial benefit of insulin treatment in T1DM was clearly shown, it was expected that it should work for T2DM as well. Since the assumed pathophysiology was similar, the expectation was strengthened. On this background, it would be natural to be reluctant to accept counterintuitive results. Confirmation bias comes out of human nature and may not be treated as a crime, but there needs to be a continued attempt to overcome it and face the reality above prior beliefs and prejudices. Going by the results of clinical trials, considering all alternative possible explanations of the results it needs to be clearly recognized that there is no conclusive evidence so far that glucose normalization reduces diabetic complications in the context of T2DM. The remark by Gill (1991) quoted at the start of this chapter is applicable even after over 30 years of research (Williams J C, 1991). To derive any conclusion from a study aimed to under T2DM a study would need to be free from these biases. An apt longitudinal study would require a cohort to be followed over a long period of time (10 years) with the data being collected regularly. The cohort would need to have individuals of both diabetic and non-diabetic people from different populations with a large population size (1000-5000 participants) to draw statistical conclusions. A baseline data collection would include anthropometry measures (body weight, body dimensions (height, waist, hip), fat percentage and distribution), basic blood tests (blood counts, liver profile, kidney profile, lipid profile, thyroid profile, blood glucose, blood insulin), vitamin deficiency, iron levels, fitness indices (balance, flexibility, muscle endurance, eye hand coordination, agility), glucose tolerance curve, brain glucose



transportation rate using MRI, flow mediated dilation (FMD) tests, levels of different growth factors in blood and a suitable test for vascular integrity such as ocular coherence tomography angiography (OCTA). A longitudinal data collection would need to be established to collect data at regular intervals, repeating core measurements and including additional markers as study progresses. Further, the statistical analysis would be predefined and determined with help of biostatisticians using corrective measures such as Bonferroni correction to deal with high dimensional data. Some exploratory statistical techniques like network analysis and machine learning could also be included in the protocol to possibly identify complex relationships between lifestyle factors, vascular function, brain function, and metabolic markers.

The failure of multiple attempts to support the hypothesis that glucose regulation can arrest complications in type 2 diabetes should be taken as decisive and the target of treating T2DM changed accordingly. Although this is being increasingly recognized (Boussageon et al., 2017) the recognition is still fragmentary. The Lancet commission on diabetes (Chan et al., 2020) clearly recommends “access to insulin, patient education, and tools for monitoring blood glucose” only in the context of T1DM now and for T2DM, instead of emphasizing on glucose regulation, it recommends addressing “diverse environmental, behavioural, and socioeconomic causes” and “sustained reduction of common cardiometabolic risk factors”. However, the changing stance in literature has not yet reflected sufficiently in clinical practice. This needs active efforts to simultaneously educate practitioners as well as common man. Treatment of T2DM is certainly going to be a case of “medical reversal” i.e. a long standing treatment recommendation based on a belief is completely withdrawn based on evidence (Herrera-Perez et al., 2019). Facilitating the process of medical reversal along with research on effective alternatives will be critical for arresting one of the greatest health concern of the present and future.

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Appendix I

The model:

Bringing in dependence in the model

We assume that there are one or more clusters of outcomes that are interdependent, rest of the outcomes are independent of each other. Dependence is brought in the model by two parameters. f = the fraction of outcomes that are completely independent d = the number of outcomes in a cluster that are interdependent. So $o(1-f)/d$ will be the number of clusters. In a further variation we can randomize the cluster size d within a limit for each cluster until all clusters add up to approximately $o(1-f)$.

The simulations first generate data just as in the baseline model for $o.f$ number of outcomes. Then one of these outcomes is selected at random. The ratio of the number of cases in the treated group by number in control group is taken as t within that cluster i.e. for the next d number of outcomes. This is repeated until the total outcomes become o .

With 200 to 500 simulation runs for every set of input parameters the resultant distributions of OR, the proportion of outcomes turning out individually significant at $p < 0.05$ and their position in the distribution is studied.

Results: Introduction of dependence increases the number of false positives, i.e. outcomes that turn out to be individually significant at $p < 0.05$. While on an average 5 % of the outcomes should turn out to be significant by chance alone, when some degree of dependence is introduced, the average is greater than 5%. In simulations without dependence, the range of outcomes individually significant by chance ranged between 1 and 9. With moderate dependence i.e. $f = 0.7$ and d ranging between 3 and 10, between 4 to 21 outcomes were individually significant. The distribution of significant outcomes was often but not always asymmetrically towards the left or towards the right. Thus even with identical parameters, two runs could have significant heterogeneity or discordance between them.

Violation of independence also causes greater shifts of the mode of the frequency distribution of OR. The probability of the observed shift in the mode of 10 % in the pooled data over all trials was 62 % in simulations using $f = .7$ and d ranging between 3 and 10. Therefore the observed shift in the mean and median by about 10 % in the clinical data cannot be said to be significant.



The parameters n , o and i within the range used in the clinical trials being considered. For f and d , there are no empirical estimates available at present. But simulations show that even for small to moderate level of dependence, i.e. f ranging between 10 and 40 and d between 3 and 10, deviation from baseline results is substantial.

The implication of the simulation results for the glucose regulation trials is that, since the assumption of having common pathophysiological pathways is compatible with our current molecular and physiological knowledge, and repetition between aggregate and single end points is obvious, the rate of false positive significance is expected to be considerably greater than 5%. Therefore significance in these trials needs to be interpreted with caution. The total number of outcomes that turned out to be individually significant in the pooled data across the 6 trials is greater than 5 %, (18.7 % including obvious duplications and 8.7 % removing obvious duplications). The 8.7 % are distributed on the two tails nearly symmetrically and this deviation from 5 % is not very large. Therefore the probability of all the significant results of the trials appearing by chance alone is not too small to reject the possibility. The observation that in some of the trials the treatment appeared more beneficial and in other it appeared to cause more harm than good can also be explained by chance alone with a reasonable probability. Since we do not have quantitative empirical estimates of the degree of dependence, we cannot calculate the probabilities exactly. But since the assumption of common pathophysiological pathways is reasonable, serious doubts are raised about the individual significance (at 0.05 level) of some of the outcomes. At present none of the results should be treated as significant.

When we bring in treatment effect, i.e. $t < 1$, the entire distribution shifts to the left. This pattern is highly consistent and the shift in the mode/median increases with decreasing t . Owing to its consistency, a shift in mode and median can be considered a more reliable indicator of treatment effect over individual significant outcomes. Simulations suggest that when the hypothesis of a common pathophysiological causal mechanism such as chronic hyperglycemia is being tested using multiple outcomes, a shift in the distribution should serve as a more reliable indicator of treatment effect than individually significant outcomes.

The simulation model code:

```
def randomrange(lower, higher):  
    return lower + random.random()*(higher-lower)
```



```
def generate_random(size):
    return [random.random() for i in range(size)]

def test_randomrange():
    irange = (0.5, 0.8)
    for i in range(100):
        r = randomrange(*irange)
    assert r > irange[0] and r < irange[1]

test_randomrange()

def generate_groups(size):
    control = generate_random(size)
    treatment = generate_random(size)
    return control, treatment

def count_of_seen(group, seen_test):
    return len([v for v in group if seen_test(v)])

def compute_odds(C, T, size):
    return T / (size - T) / (C / (size - C))

def generate_outcomes(size, num_outcomes, irange, t=1.0):
    data = []
    while len(data) < num_outcomes:
        control, treatment = generate_groups(size)
```



```
i = randomrange(*irange)

C = count_of_seen(control,lambda x: x <i)

T = count_of_seen(treatment,lambda x: x <i*t)

try:

    stat, p,dof = chi2_contingency([[C,T],[size-C, size-T]])[:3]
i = randomrange(*irange)

    odds_ = compute_odds(C,T,size)

data.append({"pvalue":p,
"odds":odds_,
"t":T/C})
exceptZeroDivisionErroras ze:
pass
returnpd.DataFrame(data)

defplot_odds(outcomes, pval=0.05):
if'significant'notinoutcomes.columns:
    outcomes['significant'] = outcomes.pvalue.apply(lambda x:f"p<{pval}"if x
<pvalelsef"p>= {pval}")
returnalt.Chart(outcomes).mark_bar().encode(
alt.X('odds', bin=alt.Bin(maxbins=100)),
alt.Y('count()'),
color = 'significant')
defget_d(count, total, drange):
    d = int(randomrange(*drange))
    remaining = total - count
if d <= remaining:
return d
```



```

else:
return remaining

defsimulation(size, num_outcomes, fraction, irange, drange):
    """
    size -> population size
    num_outcomes -> total number of outcomes
    fraction -> fraction of independent outcomes
    irange -> range from where to choose i from
    drange -> range from where to choose d from
    """
    num_indpnt_outcomes = int(num_outcomes * fraction)
    outcomes = generate_outcomes(size,num_indpnt_outcomes,irange, t=1.0)

    alloutcomes = [outcomes]
    count = num_indpnt_outcomes
    try:
    while count <num_outcomes:
        instance = random.choice(outcomes.to_dict(orient="records"))
        d = get_d(count,num_outcomes,drange)
        outcomes = generate_outcomes(size, d,irange, instance['t'])
    alloutcomes.append(outcomes)
        count += d
    exceptIndexErrorasie:
    print("Resulted in empty as all rows were ignored!")
    print(count, d,len(outcomes))
        outcomes = alloutcomes[-1]

```



```
return pd.concat(alloutcomes)

def multiplt_simultions(M, n, o, f, irange, drange):
    outcomes = []
    for i in range(M):
        sd = simulation(n, o, f, irange, drange)
        sd['simulation'] = i
        outcomes.append(sd)
    return pd.concat(outcomes)

def plot_odds_trellis_square(outcomes, pval=0.05):
    cats = [f"p<{pval}", f"p>= {pval}"]
    outcomes['significant'] = outcomes.pvalue.apply(lambda x: cats[0] if x < pval else cats[1])
    colors = ['brown', 'skyblue']
    color_scale = alt.Scale(
        domain=cats,
        range=colors
    )

    num_cols = 5 # Number of images per row
    num_rows = np.ceil(len(outcomes) / num_cols).astype(int) # Calculate the number of rows

    return alt.Chart(outcomes).mark_bar().encode(
        alt.X('odds:Q', bin=alt.Bin(maxbins=100, extent=(0,3))),
        alt.Y('count()'),
        color=alt.Color('significant', scale=color_scale,
            legend=alt.Legend(orient="left")),
```



```

column=alt.Column('simulation:N',
                  header=alt.Header(labelOrient='bottom'),
                  sort=list(range(num_rows))),# Sort by row index
row=alt.Row('simulation:N',
            header=alt.Header(labelOrient='left'),
            sort=list(range(num_cols))),# Sort by column index
).properties(
    width=100,# Adjust the width of each image
    height=100# Adjust the height of each image
)

def plot_pval_dist(outcomes, pval=0.05):
    return plot_odds_trellis(outcomes, pval).encode(
        alt.X('pvalue:Q', bin=alt.Bin(maxbins=50))
    )

df = multiplt_simultions(M=20, n=500, o=350, f=.9, irange=(0.02,0.2), drange=(3,10))
df.to_csv("simulation_data.csv", index=False)
print(df.odds.describe())
plot_odds_trellis(df)

```



CHAPTER 4

Understanding the relationship between central and peripheral glucose regulations using Streptozotocin (STZ) induced diabetes model.



4.1 Introduction:

Streptozotocin (STZ) induced diabetes in rodents is a popular model to study what is assumed to be hyperglycemia due to beta cell dysfunction. STZ was isolated from *Streptomyces achromogenes* in 1960, but the diabetogenic property was characterized only by 1969 (Junod et al., 1969), exhibiting a dose dependent beta cell specific destruction in pancreatic islets. STZ was chemically characterized as a nitrosourea analogue able to cause cell death by DNA alkylation (S. Lenzen, 2008; Mossman et al., 1985; Rossini et al., 1977). The mechanism of specific accumulation of STZ in pancreatic beta cells was not understood until late 20th century, when it was shown to be transported specifically via low affinity GLUT-2 glucose transporter (Elsner et al., 2000; Hosokawa et al., 2001; Karunanayake et al., 1976; Schnedl et al., 1994; Tjälve et al., 1976). Although STZ started as an insulin-dependent diabetic model, it became popular as a model to study various afflictions of diabetes like the hyperglycemia and different vasculopathies, which were assumed to be of same origin in both type 1 and type 2 diabetes (Ying Cheng et al., 2019; Furman, 2015; Kanamori et al., 2015; Kramer et al., 2009; Mathias Akinlade et al., 2021; Zhu et al., 2011).

In STZ treated animals the extent of hyperglycemia is primarily dose dependent, where a higher dose nearly ablates the pancreatic islets leading to an insulinopenic, hyperglycemic and ketoacidosis state, similar to insulin dependent type 1 diabetes (Sigurd Lenzen, 2017; Like & Rossini, 1976; Rossini et al., 1977). Further studies found that low doses of STZ treatment cause only mild impairment of insulin secretion with post meal hyperglycemia, similar to later stages of type 2 diabetes (Kolb, 1987; Portha et al., 1989). But a type 2 diabetes model is incomplete without insulin resistance, which was one of the differential characteristics of the disease. Around the same time, a relationship between high fat diet and development of obesity and insulin resistance was being explored (Christiansen et al., 1997; Lovejoy et al., 1998; Mayer-Davis et al., 1997). Based on these studies a high fat diet induced insulin resistance animal model was developed, which exhibited insulin resistance, obesity, hyperglycemia, but no frank diabetes (Flanagan et al., 2008; Zhao et al., 2007). So to emulate the natural history of the type 2 diabetes, researchers developed a high fat diet-STZ induced diabetes model, where insulin resistance was induced by high fat diet, and STZ was used to induce beta cell dysfunction (Furman, 2021; Gheibi et al., 2017; Prasad et al., 2022; Reed et al., 2000; Zhang et al., 2008).



Now the STZ induced diabetes rodent model system is based on the belief that it causes permanent damage to pancreatic islets (S. Lenzen, 2008). However pancreas have been shown to have regenerative properties, with cells capable of differentiation and hormone secretion (Porat et al., 2011; Wang et al., 1996). Research investigating the mechanism of STZ action on pancreatic cells and insulin secretion, further revealed that within 24-48 hours of treatment the beta cells recover almost completely, with fasting plasma insulin levels almost similar to control, but the hyperglycemia endures (Yu Cheng et al., 2015). Further earlier work in our lab had demonstrated that despite STZ induced post meal hyperglycemia, the fasting glucose levels were comparable to control animals, indicating two distinct regulatory mechanisms for fasting and post-prandial blood glucose levels, which respond distinctly to STZ treatment (Diwekar-Joshi & Watve, 2020). The dichotomy in the observations led us to question the perceived mechanism of STZ induced hyperglycemia and also its validity as a model to study disruption of glucose homeostasis via beta cell dysregulation.

An alternative possibility is raised by a set of observations. Animals undergo acute hypoglycemic phase within first few hours of STZ treatment, with some studies detailing the extent of hypoglycemia in rabbits within 9-10 hours of STZ injection (Furman, 2021; Saleem Mir et al., 2015). It can be suspected owing to the acute hypoglycaemia that there is some change in the glucose regulation system leading to hyperglycemia via neuroendocrine axis (Routh, 2002). One possibility is that after facing the acute hypoglycaemic phase, the glucose sensing neurons in the brain are either damaged or have altered responsiveness to glucose. Alternatively since STZ treatment is known to affect growth factor expression, particularly FGF (Omar et al., 2014) and FGF is important in angiogenesis (Galzie et al., 1997; Yaqoob et al., 2014), it is likely that STZ treatment affects the vasculature owing to which the glucose transport from blood to brain is slowed down. The resulting deficiency of glucose in the brain leads to hyperglycemia by neuroendocrine mechanisms. It is also known that STZ treated animals have lower GLUT1 activity across the blood brain barrier, but it is thought to be a result of hyperglycemia (Roman Duelli et al., 2000; Maher et al., 1993). It is possible that it is a cause rather than effect of peripheral hyperglycemia.

Despite the contradictory observations and advances in development of better animal models for study of type 2 diabetes, STZ induced models remain one of the cheaper, quicker and popular alternatives. Hence, we chose the STZ induced diabetes mouse model to re-examine the mechanism of STZ induced hyperglycemia. We investigated the extent of hypoglycemia



that follows STZ injections and its relation with hyperglycemia that develops later. We further planned to test the hypothesis by preventing the transient hypoglycaemia by supplementing glucose via IP (intraperitoneal) or ICV (Intracerebroventricular) pathways but this part of the work was interrupted by the pandemic.

4.2 Materials and Methods:

4.2.1 Animal model and conditions

Animals

Healthy male C57BL6 mouse of age 6 to 8 weeks weighing 20-25 grams were used throughout. For each study animals the cohorts were matched for weight and age and randomized into treatment groups.

Approval by the Ethics Committee

The experiments were approved in advance by Institutional Animal Ethics Committee at IISER, Pune (Protocol number IISER_Pune/ IAEC/2019_2/19 and IISER_Pune/ IAEC/2019_2/18) and performed at National Facility for Gene Function in Health and Disease, IISER Pune. The approval committee is constituted by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

Housing of animals

The C57BL6 mouse were housed at a temperature of $23\pm 2^{\circ}\text{C}$ with a 12-hour light/dark cycle, with standard chow (Altromin rat/mice maintenance diet) and water available ad libitum. Each cage housed 3-5 animals, with care taken to acclimatise mouse from a different birth cohort. The bedding of the cages was changed every three days till the animals were of a suitable weight for the experiment. The bedding was changed daily after the STZ injection for the duration of the experiment. There were no extra measures taken for the enrichment of the animals until mentioned otherwise.

Euthanasia

The animals were sacrificed using either carbon dioxide induced euthanasia or injection of Ketamine/Xylazine (100ul, 10:1). Liver, pancreas and brain of some animals were stored for future studies. In such cases euthanasia was induced using Ketamine/Xylazine mixture. In all



other cases euthanasia was induced by placing animals in anaerobic chambers and introducing 100% carbon dioxide at 5 liters/minute for 3 minutes. Animals were then kept under observation for another 5 minutes and death was ascertained by cardiac and respiratory arrest with fixed and dilated pupil.

4.2.2 Streptozotocin (STZ) treatment

Male C57BL6 mice of age 6 to 8 weeks, weighing 20-25gms were fasted for 6 hours before they were intraperitoneally (IP) injected with STZ at a dose of 120mg/Kg or 125mg/Kg of body weight. The doses were decided after comprehensive standardizing process. At the above mentioned dose, 100% animals survived and 80% of animals exhibited post meal hyperglycemia. Food was added immediately after the injections to the cages. Care was taken to inject the animals after 14:00 hours daytime to maximize the effect of STZ treatment and after minimum 2 hours of fasting. STZ was prepared immediately before injection by dissolving in citrate buffer (Citric Acid: 0.1M and Sodium Citrate: 0.1M, Ph 4.1). Control group was injected with matched volume of citrate buffer (CB).

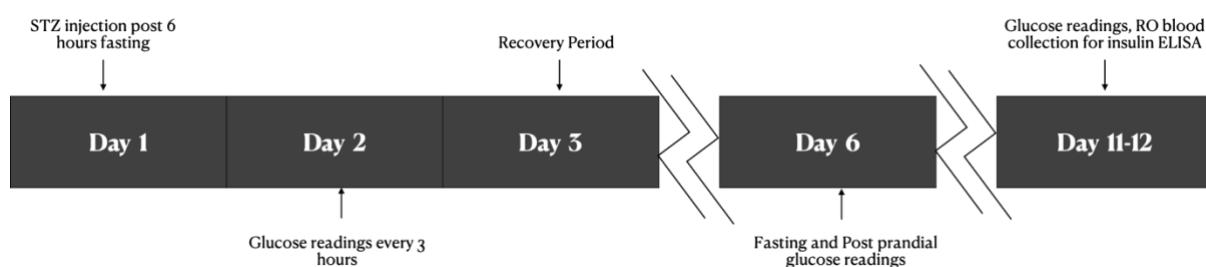


Figure 4.2: STZ treatment plan

4.2.3 Sampling for glucose estimation:

For testing the immediate effect of STZ, blood glucose reading was taken by tail nip method, using AccuChek Glucometer for glucose reading. The glucose reading was taken every 3 hours for the first 24 hours after STZ injection. To discount for the stress caused by first tail nip, the first reading was taken 30 minutes after the first tail nip. This method required only 0.5-1ul of blood, hence 7-8 readings could be taken in the first 24 hours. Fasting and perturbed glucose readings were taken 6 days, 9 days and 12 days later by tail nip method. Fasting glucose levels were measured after overnight (~16 hours) fasting and post meal glucose levels were measured 2 hours after adding food to the cages.



4.2.4 Glucose supplementation:

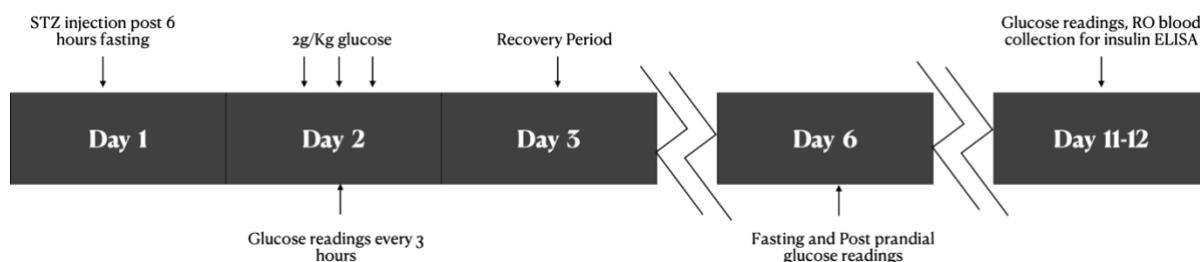


Figure 4.3: STZ treatment with glucose supplementation.

A weight and age matched cohort of STZ treated animals were intraperitoneally injected with 2g/Kg glucose at 9 hours or 12 hours or 15 hours after STZ injection in an attempt to prevent the transient systemic hypoglycaemia following STZ treatment.

4.2.5 Sampling for insulin estimation:

On 9th or 12th day after STZ injections, 300 μ L-700 μ L blood was collected via the retro-orbital sinus from the mice for the measurement of insulin using isoflurane for anaesthesia. Insulin was measured using Mercodia Mice Insulin ELISA kit (10-1247-01), with protocol followed as instructed in the kit guidelines. Because of the difficulty and stress to the animal caused by sampling, insulin estimations could not be done as frequently as glucose estimation.

4.2.6 Intracerebroventricular (ICV) infusion:

Surgical preparations

Animals were anesthetized using ketamine(80mg/Kg)/xylazine(10mg/Kg). Animals underwent stereotaxic insertion of a guide cannula in the lateral ventricle at coordinates from the bregma: -0.10 mm anterior, +0.80 lateral, -2.25 below the skull surface(Paxinos & Franklin, 2001). The ICV cannula was prepared using 35G needles as mentioned by Kokare et al (Kokare et al., 2011). Only animals that had regained preoperative body weight with no signs of infection or illness were studied 1 week later.

Intracerebroventricular infusion of glucose

Animals were IP injected with STZ (120mg/Kg) or CB, depending on the treatment group, seven days post-surgery. 6 Hours after STZ injections, animals were treated with either 2mg/Kg



IP glucose (in saline) or ICV glucose (in aCSF) infusion depending on the treatment group. Control groups were injected with volume matched artificial cerebrospinal fluid (aCSF) without glucoses (Booker et al., 2014). They were then kept under observation for three days, before proceeding to glucose sampling for fasting and post meal state.

4.2.7 Statistical analysis:

Correlation of glucose levels between different time points post STZ injections was done using Pearson's correlation coefficient r . Since data had some outliers, non-parametric Spearman's ranked correlation analysis was used to back up. Insulin and glucose levels of the control and STZ treated animals were compared using a non-parametric Mann Whitney U test.



4.3 Results

4.3.1 Acute hypoglycemia observed in streptozotocin-induced animals

A wide range of single doses of STZ have been reported for inducing hyperglycemia in C57BL6 mice. Therefore, we standardized the dose and determined the optimal range to be 120-125 mg/kg based on 100% survival and the minimum dose required for effective induction

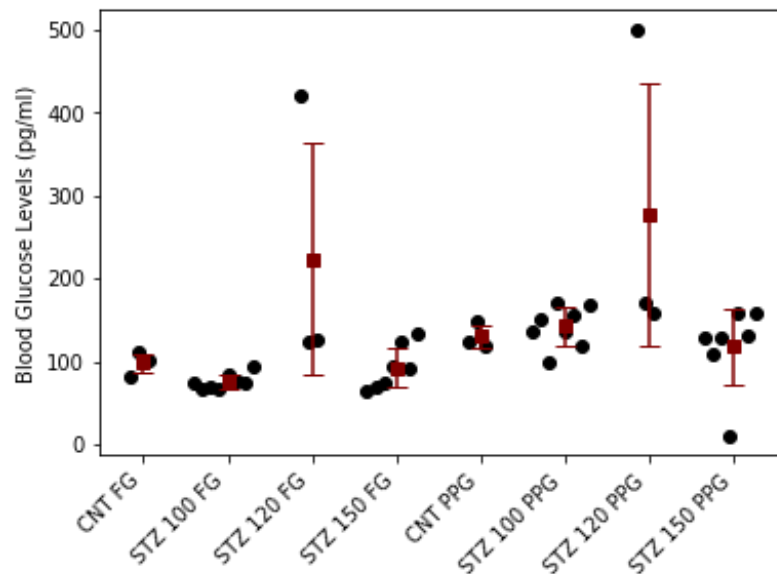


Figure 4.3: Standardization of streptozotocin dose in C57BL6. Fasting glucose (FG) and post prandial glucose (PPG) levels of animals treated with 100mg/Kg (STZ 100), 120mg/Kg (STZ 120) and 150mg/Kg (STZ 150) is compared with citrate buffer treated control (CNT). N=3 for CNT, N=8 for STZ 100, N=4 for STZ 120, N=9 for STZ 150.

of hyperglycemia (Figure 4.3).

To investigate the effects of a dose of 120-125mg/Kg of streptozotocin (STZ) on beta cell dysfunction and insulin release, we compared plasma insulin levels in control (CNT) and STZ-treated animals 12 days after STZ treatment. Although we did not quantify active beta cells, we chose to examine insulin levels as an indicator of beta cell function. To allow for stabilization, we assessed fasting and postprandial insulin levels.

Surprisingly, we found no significant differences in insulin levels between CNT and STZ-treated animals, despite the presence of persistent hyperglycemia in the latter group (Figure 4.4). These findings suggest that the reduction in insulin release and extrapolation to beta cell destruction may not be the primary mechanisms underlying the observed hyperglycemia in STZ-treated animals. While these results are unexpected, they highlight the complex



interactions between different physiological and metabolic pathways in regulating glucose homeostasis.

In order to elucidate the role of hypoglycemia in the onset of hyperglycemia following streptozotocin (STZ) administration, blood glucose levels were monitored at 3-hour intervals for 24 hours in experimental animals. The results indicate significant fluctuations in blood glucose levels, particularly during the first 3-6 hours after STZ treatment, during which there was a marked increase followed by a steep decline in blood glucose levels. These levels

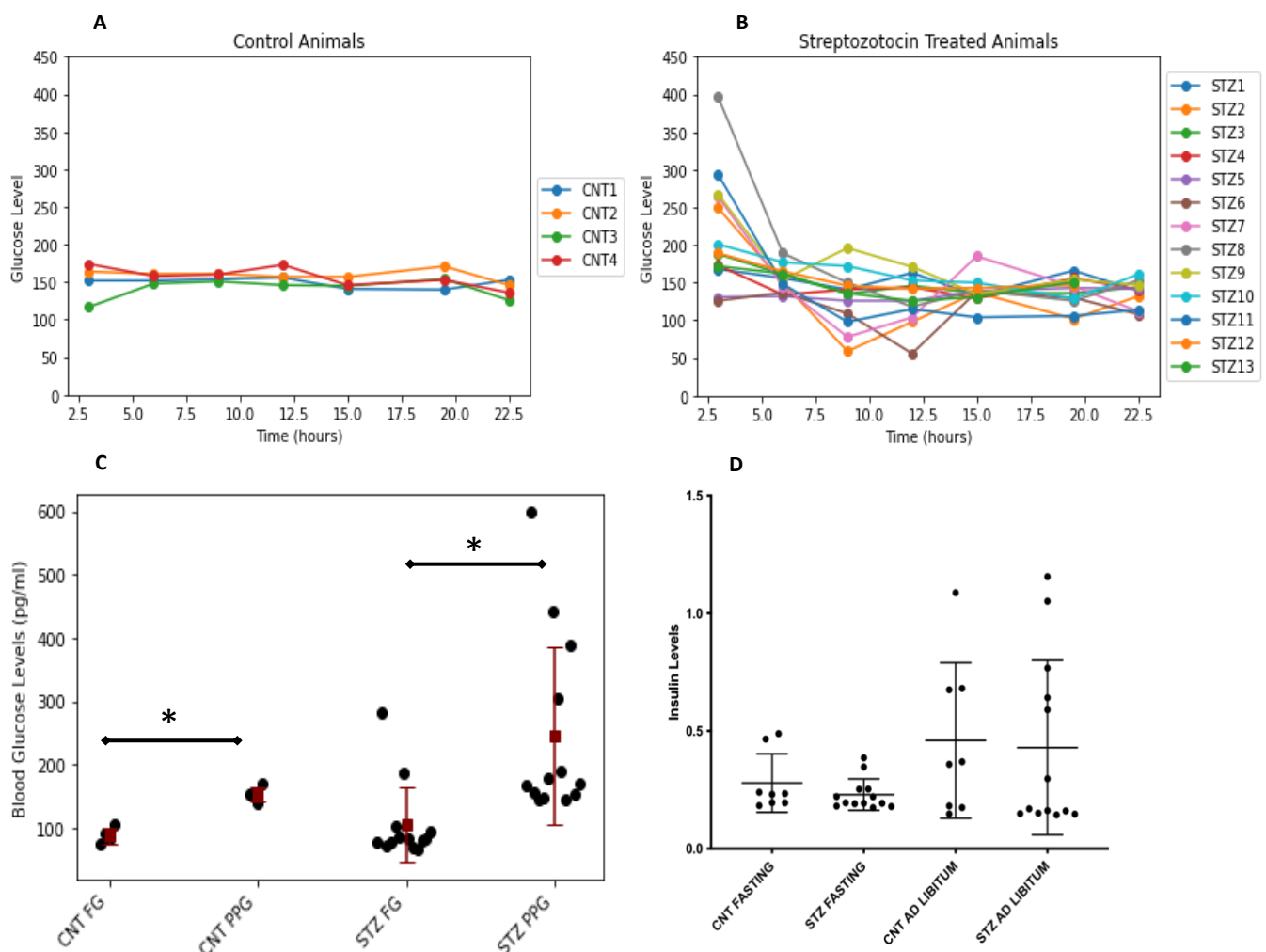


Figure 4.4: Blood glucose and plasma insulin levels in control and STZ-treated animals. A) Control animals treated with citrate buffer show low variability in blood glucose levels. B) STZ-treated animals exhibit high variability in blood glucose levels post-treatment. C) Average blood glucose levels in STZ-treated animals are significantly higher than controls. D) Insulin levels ($\mu\text{U}/\mu\text{l}$) in CNT and STZ treated animals. $N=4$ for controls and $N=13$ for STZ-treated animals. Error bars represent standard error of the mean.



continued to decrease until 12 hours post-STZ treatment before gradually returning to normal levels after 15-20 hours. Overall, STZ-treated animals exhibited considerable variability in blood glucose levels throughout the 24-hour observation period compared to the control group (Figure 4.4).



4.3.2 Correlation between fluctuating blood glucose levels post STZ treatment and

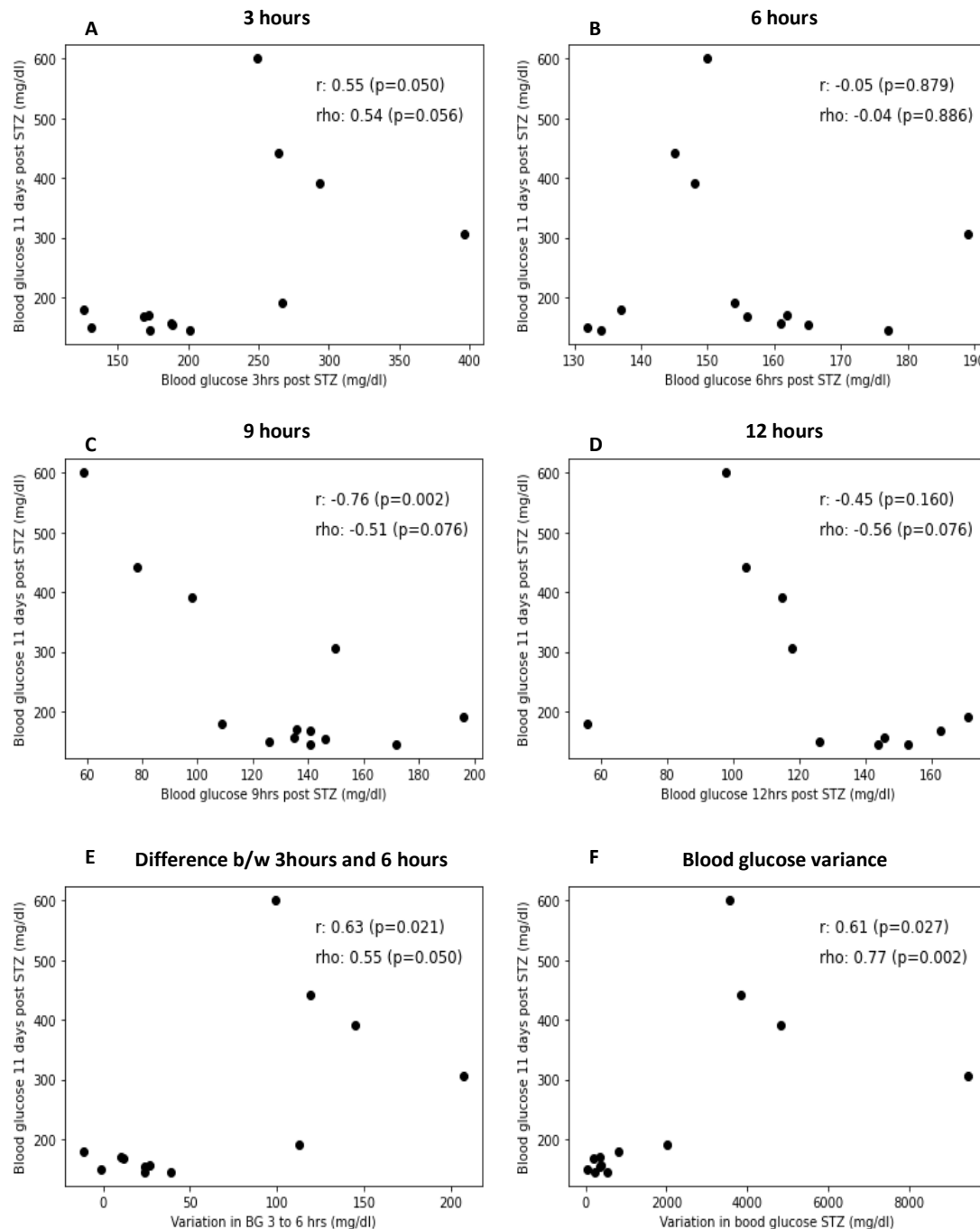


Figure 4.5: Blood Glucose Correlation Analysis: The study aimed to investigate the correlation between blood glucose levels at various intervals after STZ treatment and postprandial glucose levels at 11 days post-treatment. Correlations were analysed for blood glucose levels at 3, 6, 9, and 12 hours after STZ treatment, as well as the variability in blood glucose levels between 3 and 6 hours post-treatment. The results showed that blood glucose levels at 3 hours exhibited a positive correlation, whereas the levels at 6, 9, and 12 hours showed a negative trend with postprandial glucose levels. Among these, only the correlation between 9 hours and postprandial glucose levels was significant (A, B, C, D). Furthermore, postprandial glucose levels were found to be positively correlated with the drop in blood glucose levels from 3 to 6 hours and the variance in blood glucose levels.



development of hyperglycemia

The present study aimed to investigate the effect of hypoglycemia following STZ treatment on blood glucose levels and its potential contribution to the development of hyperglycemia. To achieve this, a correlation analysis using Spearman's and Pearson's coefficients was conducted, based on the extent of hypoglycemia observed in a group of animals within 24 hours of STZ administration.

Our results revealed an extraordinary rise in blood glucose levels 3 hours post-STZ administration, followed by a large variation within 24 hours. We observed a positive correlation for 3 hours, whereas 6, 9 and 12 hours showed negative trend, one of which was statistically significant. Further, a positive correlation was found between the drop in blood glucose levels from 3 hours to 6 hours and between blood glucose variance and post prandial glucose level (Figure 4.5). Since the acute hypoglycemic points are not synchronized in the animals, no one time point or one hypoglycemic event shows a significant correlation to the post prandial glucose levels. But this problem is overcome when a correlation analysis between blood glucose variance and post prandial glucose level is done, showing a significant positive correlation between hypoglycemia and post prandial glucose levels.

Although not conclusive, the correlation analysis shows a significant relationship between the rate of drop in blood glucose levels post-STZ treatment and post prandial glucose levels on 12th day, suggesting that the hypoglycemic episodes contribute to the development of hyperglycemia in the long term, highlighting the importance of understanding the underlying mechanisms that contribute to this phenomenon.

4.3.3 Peripheral glucose supplementation alone cannot prevent hypoglycaemia

In order to investigate the role of peripheral glucose variations and hypoglycemic episodes following STZ treatment, we attempted to mitigate their effects by supplementing with IP glucose at a dose of 2g/Kg upon detection of hypoglycemia. However, despite multiple glucose IP supplements, the treated animals continued to display hypoglycemia. As the intervention itself was unsuccessful the subsequent development of postprandial hyperglycemia was not unexpected (Figure 4.5 and 4.6). Our intended hypothesis could not be tested by this experiment.



However although the infused glucose did not prevent hypoglycemic events, it destroyed the correlation observed earlier between hypoglycemic events and post prandial glucose levels post 11 days of STZ treatment.

As IP glucose supplementation experiments in STZ treated animals led to inconclusive but suggestive relationship between hypoglycemia and post prandial hyperglycemia, we planned to test whether ICV infusion of glucose could prevent post prandial hyperglycemia in STZ treated animals.

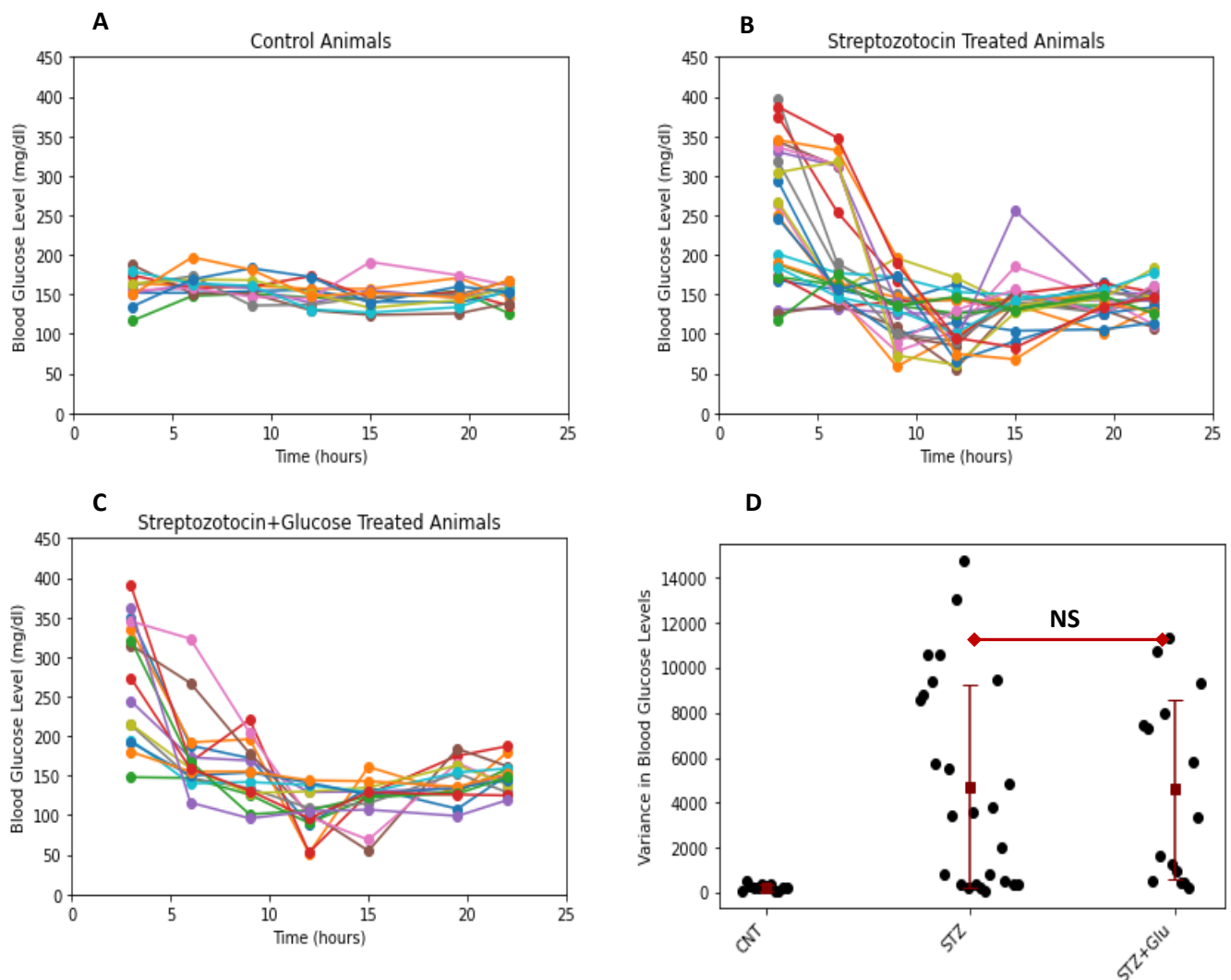


Figure 4.6: Effect of Glucose Supplementation on Hyperglycemia Development in STZ-Treated Animals. Panels A, B, and C show blood glucose levels in control animals, STZ-treated animals, and STZ-treated and glucose-supplemented animals, respectively. Panels D and E show the correlation between the variance of blood glucose levels and post-prandial blood glucose in STZ-treated animals and glucose-supplemented STZ-treated animals, respectively. Panel F shows the difference in variance in blood glucose levels up to 24 hours post-STZ treatment between the different treatment groups. $N=12$ for CNT, $N=24$ for STZ, $N=15$ for STZ+Glucose.



Blood glucose post 9 hours of STZ

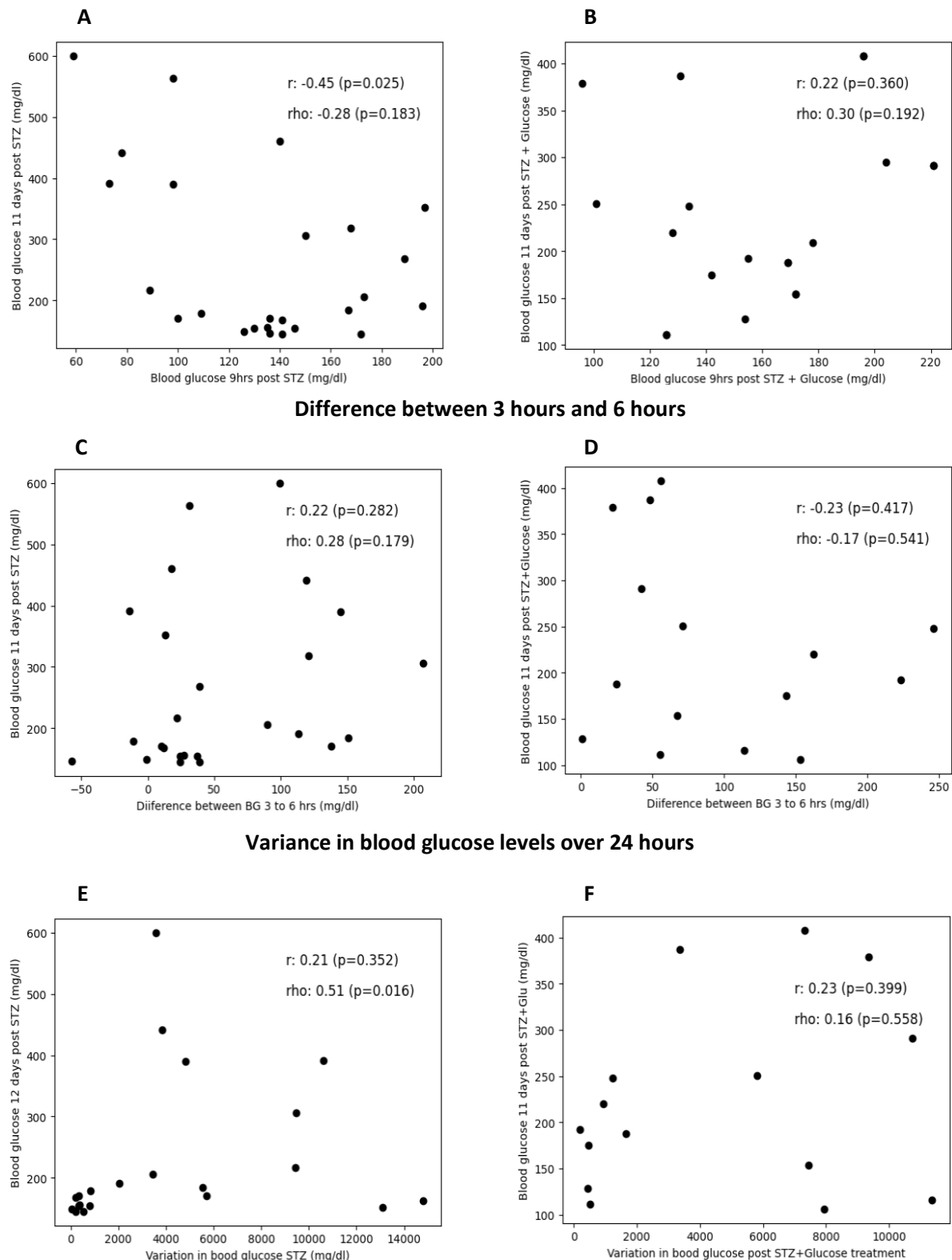


Figure 4.7: Effect of glucose infusion post STZ treatment. Glucose IP supplementations were given between 6 to 12 hours post STZ treatment, $N=24$ for STZ, $N=15$ for STZ and glucose treatment (STZ+Glu). 'r' is for Pearson's correlation coefficient, 'rho' is for Spearman's correlation coefficient.



4.3.4 Exploring the effect of central glucose supplementation on type 2 diabetes development

Having investigated various peripheral aspects of glucose regulation, which may contribute to the development of hyperglycemia via streptozotocin (STZ) treatment, we sought to explore the potential role of central mechanisms in this process. Despite observing comparable insulin levels between STZ-treated animals and controls, as well as supplementing with glucose during hypoglycemic episodes, animals continued to develop hyperglycemia.

Our previous observations suggested a relationship between the variance in glucose levels post-STZ treatment and postprandial glucose levels, indicating that hypoglycemic episodes or high variation in blood glucose levels may affect glucose-sensing neurons in the hypothalamus. As this brain region is not completely insulated by the blood-brain barrier, we hypothesized that direct infusion of glucose into the lateral ventricles of the brain via intracerebroventricular cannulation might shed light on the underlying mechanisms involved.

Unfortunately, due to continuous interruptions caused by lockdowns during the covid pandemic, we were unable to complete this line of experiments in its entirety. The limited number of experiments that were completed were insufficient to draw any significant conclusions.

4.4 Conclusions

In this study, we aimed to investigate the contribution of beta cell dysfunction in the development of hyperglycemia in type 2 diabetes using a STZ-induced mouse model, which is commonly used to study the mechanisms of diabetes. Despite contradictory reports in the literature, the hypothesis that beta cell dysfunction is the prime event leading to hyperglycemia remains to be extensively examined (Porat et al., 2011; Wang et al., 1996). Insulin levels reported in STZ animals in literature, are highly variable, with some exhibiting decreased levels of insulin and others reporting no effect, while sometimes even ignoring the statistical significance of the data (Goyal et al., 2016; Koksai, 2015). But in majority of papers on STZ treated animals, insulin levels are not reported (Cassano et al., 2020; Ying Cheng et al., 2019; Elsner et al., 2000; Jiang et al., 2021; Yang et al., 2009). Rather it is assumed that since beta



cells are destroyed, insulin would not be produced. In our experiment that was certainly not the case.

To investigate the role of central glucose regulation in the development of hyperglycemia post-STZ treatment, we planned to increase glucose levels in the brain by infusions in the lateral ventricle. Although we could not perform the planned experiments, literature evidence suggests that STZ-induced damage to central glucose regulation pathways in the brain may contribute to the development of hyperglycemia. STZ infusions in the brain have shown to lead to cognitive deficit via cell death (R. Duelli et al., 1994; Grieb, 2016; Sapcanin et al., 2008). Now STZ is specifically taken up by GLUT2 transporters which are found abundantly in hypothalamus and brain stem, areas also known for glucose sensing neurons (Grieb, 2016; Routh, 2010). We postulate that one of the ways that STZ induces hyperglycemia might be disruption of glucose sensing mechanism.

The literature suggests that STZ-induced damage to the hypothalamus, important in regulating glucose metabolism, could cause oxidative stress and inflammation in the hypothalamus, impairing the function of glucose-sensing neurons and disrupting the regulation of glucose metabolism. Additionally, STZ treatment has been shown to impair neurovascular coupling in the brain, which could contribute to the dysregulation of glucose metabolism by decreasing glucose delivery to neurons (Arrick et al., 2007; Vetri et al., 2012).

Literature review reveals that unlike the classical assumption, STZ treatment is not specific in its action on beta cells. Various effects of STZ treatment on hormones and growth factors that regulate glucose and lipid metabolism, phosphate homeostasis, cell growth and differentiation, angiogenesis, vascular remodelling, blood pressure, fluid balance, and erythropoiesis are reported (Ahn et al., 2015; Anwana & Garland, 1991; Arrick et al., 2007; M.-F. Cheng et al., 2010; Gong et al., 2013; Hicks et al., 1998; Kianifard et al., 2012; Mokuda & Sakamoto, 1999; Park et al., 1997; Schnedl et al., 1994; Sundaresan et al., 1984). STZ treatment is found to have various effects on these factors, suggesting that it may impact multiple metabolic pathways, some of which may affect glucose dynamics. Therefore STZ acts via beta cell destruction, is not a very well supported assumption.

For example, STZ treatment decreased FGF-19 expression in the liver of diabetic rats, which could affect glucose and lipid metabolism (Li et al., 2018). Similarly, STZ treatment decreased IGF-1 expression in various tissues including prefrontal cortex, which could impact cell growth



and differentiation (Aksu et al., 2012). Increased levels of FGF-23 in liver, kidney and serum have been observed post STZ treatment in rats (Dokumacioglu et al., 2019). Furthermore, STZ treatment increased FGF-21 expression in the liver and adipose tissue of rats and mice, although other studies reported decreased FGF-21 expression in the liver and plasma of diabetic rats (Degirolamo et al., 2016; Omar et al., 2014; Ying et al., 2019). FGF family growth factors are also involved in angiogenesis (Cross & Claesson-Welsh, 2001; Presta et al., 2005; Slavin, 1995) and their disruption may affect brain vasculature. It is important to note that a single FGF2 injection was sufficient for long term remission of hyperglycemia in STZ animals (Lin et al., 2016). Therefore it is quite likely that STZ induced hyperglycemia is primarily owing to its action on FGF expression.

In conclusion, although our study is short of demonstrating any alternative mechanism, it raises question on whether the beta cell destruction and resultant insulin deficiency is the only possible mechanism leading to hyperglycemia. The disruption of central glucose regulation pathways in the brain, impaired brain vasculature, problems in FGF expression and multiple impacts on various metabolic pathways may contribute to the development of hyperglycemia in the STZ induced rodent model. It is necessary therefore to re-examine the STZ model and the conclusions drawn from it so far.



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CHAPTER 5

Developing a brain centred glucose homeostasis model



5.1 Introduction

In earlier chapters we investigated several aspects of the classical hypothesis. Firstly, we examined the evolutionary role of insulin and its multi-functionality across the animal kingdom. Secondly, we analysed data from large clinical trials to test the belief that hyperglycemia is responsible for the clinical manifestations in T2DM. We found that there is no evidence that intensive glucose control impacts all-cause mortality or macrovascular events. Finally, we evaluated the role of beta cell dysfunction on hyperglycemia by conducting experiments on STZ-treated animals. We could not find conclusive proof that loss of beta cells alone is responsible for hyperglycemia, as animals showed comparable levels of plasma insulin after 12 days of treatment despite persistent post-prandial hyperglycemia.

Historically, experiments conducted during the 19th century demonstrated that damage to the fourth ventricle in the brain directly affects glucose homeostasis (Bernard, 1859). However, after the discovery of insulin and its success in treating type 1 diabetes (Banting et al., 1922) that field of diabetes research became gluco-insulin centric. The differentiation between type 1 and type 2 diabetes had not yet been established, and insulin became the primary focus of glucose regulation for almost a century (Lundqvist et al., 2019).

It was not until half a century later that the distinction between type 1 and type 2 diabetes was established, revealing that insulin deficiency was not the primary cause of hyperglycemia in type 2 diabetes. However, by now the field was primarily focused on insulin, as a consequence of which the inability of normal or increased insulin levels to regulate plasma glucose was labelled insulin resistance without considering alternative hypotheses.

The insulin resistance hypothesis has been criticized for its circular logic, which makes it unfalsifiable (Diwekar-Joshi & Watve, 2020). Insulin resistance is said to be responsible for the inability of normal or raised insulin levels to regulate glucose, but measuring insulin resistance relies on the inability of insulin to regulate glucose. There is no independent measure of insulin resistance that can be used to test its causal role. Experiments using tissue-specific insulin receptor knockouts (Blüher et al., 2002; Kadowaki, 2000), insulin-degrading enzyme knockouts (Costes & Butler, 2014; Maianti et al., 2014), pharmaceutical insulin suppression by diazoxide and octreotide (Giustina et al., 1991; Lamberts & Hofland, 2019; Leahy et al., 1994; Matsuda et al., 2002), insulin gene suppression by RNAi (Mehran et al., 2012), and alteration in insulin gene dosage (Templeman et al., 2015) do not consistently alter fasting



glucose, although they do affect the post-meal glucose curve. Epidemiological data show that fasting insulin and fasting glucose are poorly correlated, while post-meal insulin and glucose are strongly correlated, contradicting the classical models.

Based on converging evidence, Diwekar-Joshi and Watve (2020) questioned whether insulin resistance and the failure of compensatory hyperinsulinemia is sufficient to explain fasting hyperglycemia. Many mathematical models have been constructed based on the assumption of insulin resistance, with a common set of assumptions that fasting glucose is a steady state achieved by a balance between glucose uptake by tissues and glucose production by the liver. These processes are regulated by insulin signalling in the steady state. Therefore, insulin signalling has been central to these models. Recent research has exposed the inadequacies of this model (Diwekar-Joshi & Watve, 2020), highlighting the need for better alternative models.

An alternative to the classical model, which posits brain glucose level as the target of homeostasis, has been proposed and developed into mathematical models (Gaohua & Kimura, 2009; Watve, 2013). However, there has not been sufficient exploration of these models to enable comparative evaluation with peripheral models regarding their predictions and physiological and clinical implications.

In order to gain a comprehensive understanding of T2DM, we aimed to develop a glucose homeostasis model that could explain most, if not all, aberrant observations in the field. Our model differs from others in that it is thresholded, rather than consequential. Plasma glucose and insulin levels are determined by the brain's glucose and insulin requirements, rather than simply being a consequence of energy input and output reactions.

5.2 What are the essential criteria that a novel glucose homeostasis model must satisfy?

A glucose homeostasis model must be able to explain a range of experimental results beyond altered fasting steady-state glucose levels and glucose curve changes in pre-diabetic and diabetic states. A model prediction matrix is a robust and appropriate approach for comparing alternative models. This matrix tests the predictive accuracy of each model against known empirical patterns. Similar approaches have been applied successfully in other contexts (Shinde et al., 2021; Thakar et al., 2003; Vibishan & Watve, 2020; Watve & Diwekar-Joshi, 2016) and could be used to test alternative models of glucose homeostasis in T2DM. The ideal model should be capable of predicting most, if not all, of the known physiological patterns in type 2



diabetes, minimizing anomalies. We have taken a few steps towards constructing a model prediction matrix, but much more exploration of various other models is required before it can be completed.

The following is a list of established empirical patterns that a model must conform to.

1. The model must enable stable steady-state glucose levels during fasting. Fasting glucose levels are generally considered to be in a state of equilibrium, as evidenced by previous studies (Lerner & Porte, 1972; Matthews et al., 1985; R. C. Turner et al., 1979). Achieving a stable steady-state is a fundamental objective of all homeostasis models, and many of the fundamental concepts in classical models are based on achieving equilibrium during fasting (Matthews et al., 1985; R. C. Turner et al., 1979).
2. In addition, the model must permit stable steady-state insulin levels during fasting. While this requirement is typically not problematic for most models, some models struggle to account for the changes in steady-state insulin levels observed in prediabetic conditions, as elaborated below.
3. A prediabetic state is often characterized by hyperinsulinemia and normoglycemia, with high levels of fasting insulin (FI) but normal fasting glucose (FG) in the plasma. Achieving this state in a model presents a significant challenge. The classical explanation for this state is that it reflects a condition of insulin resistance with compensatory increases in FI to maintain normal FG. To accomplish this, the level of insulin resistance must be estimated and insulin secretion must be regulated accordingly. A glucose-dependent or glucose-independent mechanism for sensing insulin resistance and communicating this information to the pancreas may be hypothesized. However, no glucose-independent mechanism for accurately assessing insulin resistance and conveying it to β cells is currently known.

One common assumption is that insulin resistance leads to reduced glucose uptake, which in turn increases FG. This rise in FG then stimulates insulin production, which normalizes glucose levels by increasing insulin levels. However, this mechanism fails to establish a steady-state hyperinsulinemic normoglycemic condition (Diwekar-Joshi & Watve, 2020). Since plasma insulin has a short half-life, it is unlikely to remain elevated after glucose levels have been normalized unless there is a glucose-independent trigger for insulin secretion. Thus, explaining a hyperinsulinemic normoglycemic steady state poses a challenging problem that any model must address.



4. Hyperinsulinemia is a condition in which there is excess insulin in the bloodstream, and it is commonly believed to be a compensatory response to insulin resistance, which is a characteristic of type 2 diabetes mellitus (T2DM). However, several studies have shown that hyperinsulinemia precedes both obesity and insulin resistance (Corkey & Shirihai, 2012; Mehran et al., 2012; Pories & Dohm, 2020; Shanik et al., 2008; Weyer et al., 2000; Wiebe et al., 2021). This raises two important and independent questions: first, how and why hyperinsulinemia occurs before insulin resistance; and second, if hyperinsulinemia is not a compensatory response to insulin resistance, then the traditional explanation of compensation becomes redundant. Therefore, it is necessary to provide a more coherent explanation of the underlying mechanisms that contribute to hyperglycemia in these cases.
5. The model must be able to account for the various characteristics of the post-meal glucose curve, as well as its alterations in prediabetic and diabetic states, which together are known as the impaired glucose tolerance curve. Specifically, the model must explain the increased height of peaks, the delayed return to normal, and the increased insulin response lag in the diabetic state.
6. The state of normal fasting glucose with impaired glucose tolerance (NFG-IGT) is a prediabetic condition in which the individual's glucose tolerance curve is altered despite having a normal level of fasting glucose. This condition is characterized by a delayed return to normal glucose levels after a meal and an increase in the time difference between the glucose and insulin peaks. NFG-IGT is an important early marker of impaired glucose regulation and is associated with an increased risk of developing type 2 diabetes and cardiovascular disease. Therefore, any alternative model of glucose homeostasis must be able to accurately explain and simulate this condition.
7. Stress-induced hyperglycemia: A model that provides realistic and testable assumptions is required to explain why and how stress leads to hyperglycemia only in certain individuals (Dungan et al., 2009).
8. Exercise-induced hyperglycemia: While fasting, the level of plasma glucose is believed to be solely determined by the rate of glucose disposal and liver glucose production. Exercise typically increases glucose disposal, which should lead to a decrease in fasting glucose levels. However, some studies have shown that plasma glucose remains unchanged or even increases after exercise (Coggan, 1991; Marliss & Vranic, 2002). A model that accounts for this phenomenon is necessary.



9. An important challenge is to explain why knockouts of insulin receptors specific to muscle, fat cells, or liver result in normal fasting glucose levels but altered post-glucose load curves. Additionally, insulin-degrading enzyme knockouts do not affect fasting glucose levels. Moreover, agents like octreotide or diazoxide, which suppress insulin, fail to alter fasting glucose levels (Diwekar-Joshi & Watve, 2020). A model should be able to explain these observations. Diwekar-Joshi and Watve (2020) differentiated between the consequential steady-state (CSS) and targeted steady-state (TSS) models. In a consequential steady-state (CSS) model the glucose level is achieved as a balance between production and utilization rates, whereas in a targeted steady-state model (TSS), the glucose level is actively monitored and controlled by turning on mechanisms when it crosses a threshold. It was demonstrated that the results of the insulin receptor knockout and insulin suppression experiments can be explained by a TSS but not by a CSS model. Therefore, to account for these results, a model needs to be a TSS model (Diwekar-Joshi & Watve, 2020).
10. Reduced glucose transport to the brain, particularly in obese or prediabetic individuals, has been observed for a long time. It is known that in type 2 diabetes, the rate of glucose transport from the blood to the brain is significantly reduced (Gjedde & Crone, 1981). Studies on rodent models have shown a reduction in GLUT1 expression in brain capillaries (Cornford et al., 1995; Matthews et al., 1985; Pardridge et al., 1990). While this is hypothesized to be a response to hyperglycemia, Hwang et al. (2017) demonstrated that subnormal transport is evident in the obese and presumably prediabetic state even before hyperglycemia appears. A model needs to account for this pattern as a causal or consequential phenomenon.
11. The deposition of islet amyloid specific to T2DM which often accompanies islet deterioration: Islet amyloid deposition is a common feature of type 2 diabetes but is not observed in type 1. The causes of islet amyloid deposition should be consistent with the glucose homeostasis model (Höppener et al., 1999; Porte & Kahn, 1989; Watve et al., 2014).
12. Beta (β) cell deterioration pattern: In T2DM, the deterioration of β cells follows a distinct pattern, in which a significant proportion of β cells survive throughout an individual's life (Bacha et al., 2013; Butler et al., 2003; Clark et al., 2001; Porte & Kahn, 2001; Watve et al., 2014). If a model assumes that dysfunction and damage to the β cell



population are essential prerequisites for hyperglycemia, then it needs to account for the specific population dynamics of β cells observed in T2DM.

13. Over the last decade, SGLT2 inhibitors have emerged as a novel means of combating hyperglycemia by allowing greater clearance of glucose through urine. However, the lowering of plasma glucose by SGLT2 inhibitors is accompanied by increased liver glucose production as well as ketogenesis (Limenta et al., 2019; Mistry & Eschler, 2021; Op den Kamp et al., 2021; Pfützner et al., 2017). Therefore, any model that seeks to explain the mechanism of action of SGLT2 inhibitors should account for the increased liver glucose production and ketogenesis observed with their use.
14. In instances of brain injury, hyperglycemia is frequently observed (Shi et al., 2016). Bacterial meningitis, on the other hand, is frequently characterized by hyperglycemia along with low cerebrospinal fluid (CSF) glucose levels (Krishnan et al., 2016; Schut et al., 2009). Therefore, a comprehensive model should address this apparent discrepancy.
15. In population data, a strong correlation is observed between postprandial glucose and insulin levels, whereas a weak correlation is noted between fasting glucose and fasting insulin levels (Diwekar-Joshi & Watve, 2020).
16. Despite the weak correlation between fasting glucose (FG) and fasting insulin (FI) in the prediabetic state, population data reveals a strong positive correlation between Homeostatic Model Assessment of Beta-cell function (HOMA- β) and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) (Diwekar-Joshi & Watve, 2020).
17. In contrast to type 1 diabetes mellitus (T1DM), numerous large-scale clinical trials have failed to consistently demonstrate a reduction in mortality with tight regulation of plasma glucose in type 2 diabetes mellitus (T2DM) (DeFronzo et al., 2015; Diabetes Prevention Program Research Group, 2015; Investigators, 2009; King et al., 1999; Klein, 2010; Lee et al., 2021; Schwartz & Meinert, 2004; R. Turner, 1998; UK Prospective Diabetes Study (UKPDS) Group, 1998). If hyperglycemia is indeed the primary pathology of T2DM, then prevention or correction of hyperglycemia should have significantly reduced the frequency of complications and mortality.
18. Irrespective of the etiology of hyperglycemia, FGF21 administration via a single injection was able to achieve sustained normalization of plasma glucose levels in all rodent models of diabetes (Laeger et al., 2017). This presents a potential anomaly that a model must resolve.



Although many models aim to explain the first, second, and fifth items on the list above, they either fall short in explaining or fail to address the remaining items. Therefore, a satisfactory model is expected to inherently predict these outcomes or be compatible with them. If an additional explanation that does not contradict the model is able to account for the phenomenon, the model can be deemed compatible. While compatibility with empirical findings cannot serve as proof or validation of the model, if a model contradicts one or more of the empirical findings, it becomes a significant reason for rejecting the model. In our subsequent description of the model results, we will use the numbers denoted to above points to indicate that the model has accounted for the particular pattern.

5.3 Various glucose homeostasis models

5.3.1 *Peripheral glucose homeostasis model*

There exists a rich history of developing mathematical models to explain glucose homeostasis, as well as the onset and progression of both type 1 and type 2 diabetes (as reviewed by (Ajmera et al., 2013; Mari et al., 2020)). However, the field's focus is heavily centered on peripheral mechanisms, such that even the review by Mari et al. (2020) neglects to cite models that incorporate the brain's role. Ajmera et al. (2013) briefly mention the Gouhua et al. (2009) model but do not expand upon its potential implications. One class of models endeavours to capture glucose homeostasis at the systems level, while other models examine individual components such as insulin-dependent glucose uptake, liver glucose production, β -cell dysfunction, glucose-stimulated insulin secretion, and incretin effects in greater detail. Nonetheless, these models have yet to account for several of the patterns listed above. These models share a central assumption that is more or less invariant, revolving around insulin resistance and the failure of insulin response to adequately compensate for it (Mari et al., 2020). Diwekar-Joshi and Watve (2020) have claimed that any model with this set of assumptions is not compatible with empirical patterns 3, 4, 6, 9, 16, and 17 listed above. Whether any variation of these models can do so remains inadequately explored. Moreover, all peripheral regulation models are CSS models, such that the fasting steady state is a consequence of the rate of liver glucose production and a concentration-dependent rate of glucose uptake. In these models, any changes to either or both of these rates inevitably alter the steady state. This contrasts TSS models, in which changes to these rates alter the time required to reach a steady state but do not alter the



steady-state glucose level (Diwekar-Joshi & Watve, 2020). To the best of our knowledge, no attempts have been made to develop a TSS model with solely peripheral mechanisms.

There is a rich history of the development of mathematical models of glucose homeostasis and the origin and progression of type 1 and type 2 diabetes. Ajmera et al. (2013) and Mari et al. (2020) have reviewed these models extensively. However, the focus of the field is mainly on peripheral mechanisms, and some reviews do not consider the role of the brain in glucose regulation. Gouhua et al.'s (2009) model is briefly mentioned by Ajmera et al. (2013) but not discussed in detail.

A class of models attempts to capture glucose homeostasis at the systems level, while others examine individual components such as insulin-dependent glucose uptake, liver glucose production, β cell dysfunction, glucose-stimulated insulin secretion, and incretin effects in greater detail. Nevertheless, these models have not accounted for many of the anomalous empirical findings, such as patterns 3, 4, 6, 9, 10, 11, 12, 13, and 15-18 listed above.

The central assumption of these models is that insulin resistance and the failure of insulin response to adequately compensate for it are the main contributors to diabetes progression. However, Diwekar-Joshi and Watve (2020) argue that any model with these assumptions is incompatible with the aforementioned empirical patterns. The potential for variations of these models to address the anomalous patterns has not been adequately explored.

Moreover, all the peripheral regulation models are classified as CSS models. This classification means that the fasting steady state is a consequence of the rate of liver glucose production and a concentration-dependent rate of glucose uptake. In these models, changes in either or both of these rates inevitably alter the steady state. This contrasts with TSS models, in which alterations in these rates change the time required to reach a steady state but not the steady-state glucose level.

We do not intend to review glucose homeostasis models here. However, we observe from published reviews of glucose homeostasis models (Ajmera et al., 2013; Mari et al., 2020) that most models do not address the apparently anomalous empirical findings particularly 3, 4, 6, 9, 10, 11, 12, 13 and 15-18 among the ones listed above. A detailed exploration into variations of each of these models and their ability to explain the aberrant observations is a possibility in future but not within the scope of this chapter.



5.3.2 Brain Glucose Homeostasis Model

It is widely recognized that numerous neuronal mechanisms in the brain are involved in energy homeostasis. However, for inexplicable reasons, they are not given central consideration in mainstream thinking regarding type 2 diabetes (T2DM) and glucose regulation models. Recent publications have highlighted the role of the brain in various ways, including those by Lam (2005), Osundiji et al. (2012), Perrin et al. (2004), Watve (2013), and Lundqvist et al. (2019).

Peters (2004), Gaohua and Kimura (2009), and Watve (2013) have proposed that the primary objective of glucose homeostasis is to regulate glucose levels in the brain. Plasma glucose levels serve merely as a means of ensuring a required supply of glucose, mainly to the brain. Because the transport of glucose to the brain is more restricted, ensuring adequate glucose supply to the brain is likely to also ensure sufficient supply to other organs.

In the Gaohua and Kimura (2009) model as well as in the Watve (2013) model, the rate of glucose transport across the BBB is assumed to be an adaptation to hyperglycemia. The finding that glucose transport is reduced to an intermediate level in obese and prediabetic individuals (Büsing et al., 2013; Hwang et al., 2017) suggests that the deficiency in transport precedes hyperglycemia rather than following it. Therefore, it is more likely to be causal than consequential. Glucose deficiency in the brain is known to induce liver glucose production and suppresses insulin release through autonomic control (Boland et al., 2017). Sympathetic tone has also been shown to be higher in T2DM (Thackeray et al., 2012). Therefore, glucose deficiency in the brain owing to altered vascular function is likely to be primary which results in altered plasma glucose dynamics mediated by autonomic inputs. Our model incorporates this possibility.

Impaired vascular function is known to be central to diabetic complications. The classical thinking has been that hyperglycemia causes the types of vasculopathies typical of T2DM. However, it has not been ruled out that vasculopathies are not primary. There is considerable evidence that microvascular alterations precede T2DM and are good predictors of its development (Muris et al., 2012; Nguyen et al., 2007; Stehouwer, 2018; Zaleska-Żmijewska et al., 2017). Sedentary life style and deficiency of many specific types of physical activities and behaviours alter the expression of growth factors and endocrine mechanisms involved in angiogenesis (Watve, 2013). It has been demonstrated repeatedly that many growth factors and angiogenic factors are responsive to behaviours, physical activity and exercise (Aloe et al.,



1994; Cao et al., 2010; Chodari et al., 2016; Lakshmanan, 1986; Nexø et al., 1981, 1984; Tirassa et al., 2003). Deficiency of these behaviours is likely to lead to a primary deficiency of angiogenic mechanisms (Watve, 2013). We consider the possibility that altered vascular function in the brain is the primary reason why glucose transport from blood to brain is reduced. This creates chronic glucose deficiency in the brain to which the brain reacts by influencing multiple mechanisms of glucose regulation. The predominant mechanism is autonomic signalling. It is well known that sympathetic and parasympathetic tones are altered in T2DM (Thackeray et al., 2012). We consider the possibility that altered autonomic signalling is causal rather than consequential to diabetes, based on studies showing that changes in autonomic function precede T2DM (Carnethon et al., 2003).

The brain glucose uptake is insulin independent (Gray et al., 2014; Hasselbalch et al., 1999). Nevertheless insulin has many other functions in the brain and insulin signalling is known to be important in memory, cognition, decision making and behaviour (Shemesh et al., 2012; Mark W. J. Strachan, 2003). Brain is rich in insulin receptors and activation of certain cognitive functions in the brain is a likely cause of increased brain glucose utilization by insulin stimulation (Bingham et al., 2002; Rebelos et al., 2021). We assume therefore that brain has a fundamental requirement for insulin, independent of glucose metabolism and therefore mechanisms to ensure the required insulin levels in the brain are as much critical as ensuring minimum brain-glucose level. This is also ensured by autonomic mechanisms. Autonomic inputs are known to regulate β cell population and as well as insulin release from β cells (Thorens, 2015). Therefore, our model also incorporates brain control over insulin production.

Ensuring minimum supply of glucose as well as insulin to the brain is crucial during fasting when the plasma levels are low. The post meal levels of glucose and insulin in the plasma are much higher and at this time, the brain need not actively regulate the plasma levels of both. This can be taken care of by the peripheral mechanisms.

5.4 Objectives of the model

Our attempt is to construct a model whose predicted outcomes match with all or most of the observed phenomena listed above, qualitatively. We intend to construct a model in which different hypotheses for hyperglycemia can be used to make differential testable predictions. This approach can allow us to evaluate comparatively which causal factors individually or in combination can give us the set of predictions that match with the patterns listed above.



Out of the parameters required for the model, only some have empirical estimates available (table 1). In the absence of realistic estimates of all parameters, we do not intend to make a model making quantitative predictions. When a sufficiently large number of parameters can be optimized, it is not difficult to fit the data quantitatively. Therefore, we prefer qualitative predictions over quantitative ones. We test whether the model is able to predict the pattern observed empirically under some set of parameters. The ability to predict a given pattern is not a proof of the validity of the model, but the inability to predict an observed phenomenon at any set of parameters in a realistic range is a strong reason to call the model either inadequate or wrong. If an additional consideration compatible with the model is able to explain a pattern not explained by the main model, the model can be called inadequate but not falsified. However, if the model outcome directly contradicts a consistent and reproducible empirical finding, it can be considered falsifying evidence. We emphasize the need to evaluate our model in comparison with classical models on these lines.

5.5 Methodology

5.5.1 Assumptions of the model

A unique assumption of our model based on the analysis by Diwekar-Joshi and Watve (2020) is that the mechanism of regulation of glucose and insulin is different in the steady state and post meal state. In the steady state the central mechanisms are more important whereas in post meal state mainly the peripheral mechanisms are at work. Insulin induced glucose uptake and insulin dependent inhibition of liver glucose production happen only above a threshold glucose and insulin level respectively. The presence of such thresholds and their context dependent variability has been known for a long time (Chen et al., 1993; Henquin et al., 2006; John Thomas Sorensen et al., n.d.). Below the threshold, other mechanisms regulate the levels (Sorenson et al., 1987). We assume in the model that the thresholds are under neuro-endocrine control and fine-tuned by the brain. The thresholds found in isolated cell cultures are slightly lower than the normal fasting blood sugar and it is difficult to estimate thresholds in vivo (Chen et al., 1993; Henquin et al., 2006, 2015). In presence of autonomic signals, we assume the thresholds to be above the steady state target levels. In conditions under which the fasting levels need to increase, we assume the thresholds to increase proportionately. This may happen with peripheral or central mechanisms. These assumptions about the threshold make the model a TSS model.



The steady state glucose is decided by a balance between basal level of liver glucose production which is regulated by neuronal mechanisms and insulin independent glucose uptake by tissues. The steady state insulin level is decided by basal rate of glucose independent insulin production, which is regulated by neuronal mechanisms against the insulin degradation rate. Glucose is transported from plasma to brain by facilitated diffusion proportionate to capillary surface area in the brain and GLUT1 expression. Steady state brain glucose is determined by glucose transport to brain and utilization by brain tissue. Brain needs a target level of glucose and the brain ensures it by directly regulating liver glucose production by neuronal mechanisms.

Insulin has cognitive as well as other functions in the brain (Begg & Woods, 2013; Kern et al., 2001; Rohner-Jeanrenaud & Jeanrenaud, 1983; Shemesh et al., 2012; M. W. J. Strachan, 2005), for which the brain requires a target level of insulin, which it ensures by autonomic regulation of β cell number and basal insulin secretion independent of glucose. For ensuring the brain target level for insulin, a minimum plasma insulin level needs to be maintained. Maintenance of this level is assumed to be independent of glucose stimulated insulin secretion.

The rate of transport of glucose and insulin from blood to brain is not constant. Both are affected by the capillary density and blood flow. But they also depend upon specific transport mechanisms. Glucose is transported across the blood brain barrier mainly through the specific transporter GLUT1. The expression of GLUT1 in brain and other tissues is variable and dependent on multiple dietary, endocrine and growth factor related mechanisms (Boado et al., 1994; Ge et al., 2011; Liu et al., 2018; Schöler et al., 2018). Glucose transport from blood to brain is diminished in obesity and prediabetes (Hwang et al., 2017). Insulin transport to brain is also reduced in obesity (Begg, 2015). The ratio of CSF to plasma insulin is inversely proportionate to obesity and insulin resistance (Gray & Barrett, 2018). More over inducing endothelial dysfunction and reducing glucose transport experimentally by endothelial specific deletion of HIF 1- α resulted into hyperglycemia (Huang et al 2012). Based on such multiple lines of evidence we incorporate the possibility of diminished glucose and insulin transport to brain being the primary pathology of T2DM and hyperglycemia only a consequence.

5.5.2 The brain targeted steady state model



Since we assume that the steady state and post meal mechanisms of glucose regulations are not identical, we model and analyse the two states in two phases of the model. The variables and parameters used in the model and the range of parameter used are in table 1.

S. no	Variables and parameters	Meaning	Units	Value/range used in simulations
A. Input parameters				
1	K_1	Insulin independent glucose uptake maximum	mg/dL/min	20-50
2	K_{1m}	Half saturation constant for insulin independent glucose uptake	mg/dl	50
3	K_2	Maximum insulin induced suppression of PG , including glucose disposal and suppression of L .	per $\mu\text{U/mL}$	0.001 to 0.05
4	K_{2m}	Half saturation constant for insulin induced suppression of PG	$\mu\text{U/mL}$	15
5	K_5	Maximum glucose dependent insulin secretion	$\mu\text{U/mL/min}$	2-20
6	K_{5m}	Half saturation constant for glucose induced insulin secretion	mg/dL	50



7	K_8	Maximum rate of glucose transport to brain	Mg/dL/min	3 - 8
8	K_{8m}	Half saturation constant for glucose transport to brain	mg/dL	50-100
9	K_9	Maximum rate of Insulin transport to brain	μ U/mL/min	0.6 - 1
10	K_{9m}	Half saturation constant for insulin transport to brain	μ U/mL	0.3-1
11	K_{10}	Brain glucose utilization rate	unitless	0.08 to 0.12
12	d_1	Insulin degradation rate	unitless	0.1 to 0.2
13	BG_t	Target BG	mg/dL	25
14	BI_t	Target BI	μ U/mL	1-5
15	I_s	Insulin sensitivity as compared to normal which is assumed 1.	unitless	0-1
16	L_{max}	Maximum capacity of liver glucose production	Mg/dL/min	30
17	K_{4max}	Maximum capacity of glucose independent insulin secretion	μ U/mL/min	10
B. Derived variables				
18	L	Rate of liver glucose production	mg/dL/min	Eq. 6 or iterative



19	K_4	Rate of glucose independent insulin secretion	$\mu\text{U/mL/min}$	Eq 7 and 8 or iterative
C. Outcome variables				
20	PG	Plasma glucose	Mg/dL	dynamic
21	PI	Plasma insulin	$\mu\text{U/mL}$	dynamic
22	$\underline{PG}$	Steady state plasma glucose	mg/dL	Eq. 5
23	$\underline{PI}$	Steady state plasma insulin	$\mu\text{U/mL}$	Eq. 7
24	BG	Brain glucose	mg/dL	dynamic
25	BI	Brain insulin concentration	$\mu\text{U/mL}$	dynamic
26	PGT	glucose threshold above which GSIS begins	mg/dL	5 to 10 mg/dL above $\underline{PG}$
27	PIT	Insulin threshold above which insulin induced glucose disposal begins	$\mu\text{U/mL}$	2-5 $\mu\text{U/mL}$ above $\underline{PI}$

Table 5.1: all variables and parameters considered in the model.

5.5.2a Modelling steady state glucose and insulin dynamics:

As per the assumption, below a threshold PI , insulin induced glucose uptake and insulin induced inhibition of liver glucose production is negligible. Therefore, we write a conditional equation such that, if the condition in the right hand bracket is satisfied, the corresponding equation on the left applies.



1. Plasma Glucose:

$$\frac{dPG}{dt} = \begin{cases} L - \frac{K_1 * PG}{K_{1m} + PG}, + Gt & (PG < PIT) \\ L - \frac{K_1 * PG}{K_{1m} + PG} + Gt - \left(I_s * \frac{K_2 * PI}{K_{2m} + PI} \right) * PG, & (PG > PIT) \end{cases} \quad \text{Eq. 1}$$

Similarly, for plasma insulin,

2. Plasma Insulin:

$$\frac{dPI}{dt} = \begin{cases} K_4 - (d1 * PI), & (PG < PGT) \\ K_4 - d1 * PI + \frac{K_5 * PG}{K_{5m} + PG} * (PG - PGT), & (PG > PGT) \end{cases} \quad \text{Eq. 2}$$

3. Brain glucose:

$$\frac{dBG}{dt} = \frac{K_8 * (PG - BG)}{K_{8m} + (PG - BG)} - K_{10} * BG \quad \text{Eq. 3}$$

Since brain forms only about 3 % of total body mass, we assume plasma glucose diffused to the brain is a negligible fraction of total plasma glucose. Therefore, that term is not included in the plasma glucose dynamics. Similarly, insulin diffusion to brain is not represented in the plasma insulin dynamics. Nevertheless, the rate of plasma to brain diffusion is crucial in determining the brain glucose and insulin dynamics.

4. Brain insulin:

$$\frac{dBI}{dt} = \frac{K_9 * (PI - BI)}{K_{9m} + (PI - BI)} - d_1 * BI \quad \text{Eq. 4}$$

At steady state (SS) all differential terms become zero and steady state levels can be calculated using equilibrium solutions.

As per the assumption of the model the brain needs a certain level of glucose and that is the target of homeostasis BG_t . In order to ensure BG_t at given K_8 , K_{10} and K_{8m} , the SS plasma glucose PG needs to be

$$\underline{PG} = \frac{BG_t * K_{10} * K_{8m}}{K_8 - (BG_t * K_{10})} + BG_t \quad \text{Eq. 5}$$

We assume that the brain ensures this plasma glucose level by regulating liver glucose production L . To maintain the desired plasma glucose level at steady state, the liver glucose production should be:



$$L = \frac{K_1 * PG}{K_{1m} + PG} \quad \text{Eq. 6}$$

The brain can either ensure this by sending a calculated signal to the liver through the autonomic system. Alternatively, this can be ensured by the brain by increasing sympathetic inputs when $BG < BGt$ and parasympathetic when it is above target (Antuna-Puente et al., 2009; D'Alessio et al., 2001; Kiba, 2004). The required L can be achieved iteratively by

$$L_{(t+1)} = L_{(t)} + K_s \cdot (BGt - BG).$$

Similarly, to ensure required brain insulin levels, plasma insulin should be

$$\underline{PI} = \frac{d_1 * BIt * K_{9m}}{K_9 - (d_1 * BIt)} + BIt \quad \text{Eq. 7}$$

To ensure this much plasma insulin level, K_4 should be

$$K_4 = d_1 \cdot \underline{PI} \quad \text{Eq. 8}$$

Similar to glucose, the brain can ensure the target level of insulin in the brain by neuronally modulating K_4 in a calculated or iterative manner. Specific autonomic inputs are known to regulate β cell number as well as insulin release from β cells (Begg & Woods, 2013; Boland et al., 2017; Kiba, 2004).

We also assume that both L and K_4 have a fixed upper limit as L_{max} and K_{4max} when the maximum capacity of liver and pancreas is reached. L and K_4 cannot exceed this limit even if neuronal inputs become more intense. This happens when K_8 declines below a threshold such that,

$$K_8 < \frac{K_1 - L_{max}(BGt * K_{10} * K_{8m})}{L_{max} * K_{1m} - BGt(K_1 - L_{max})} - (BGt * K_{10}) \quad \text{Eq. 9}$$

There is a potential conflict here. Sympathetic tone is known to increase L (Nelles et al., 1996; Nonogaki, 2000) whereas parasympathetic signal is required for proliferation of β cells. Simultaneously sympathetic signal is known to inhibit insulin release from β cells (Gilon & Henquin, 2001; Miller et al., 1976). If the brain is deficient in glucose as well as insulin, both sympathetic and parasympathetic tones will be simultaneously higher (Watve et al., 2014). This is incorporated into the model by updating K_4 iteratively according to both BG and BI levels simultaneously,



$$K_4(t+1) = K_4(t) + K_p(BIt - BI) - K_s(BGt - BG) \quad \text{Eq. 10}$$

By this consideration if sympathetic stimulation of liver glucose production is adequate to restore required BG , $BGt - BG$ will tend to zero and there will be no interference in insulin release by β cells. However, in case the rise in liver glucose production is inadequate to ensure required BG , K_4 will be affected which will apparently influence β cell function.

5.5.2b Simulating glucose tolerance curve:

While for the steady state, equilibrium solutions can be algebraically derived, the post glucose load curve and its properties can only be obtained by simulations. Simulations were run using the same set of equations and giving a positive ephemeral Gt to simulate food intake.

In all the simulations used, insulin resistance can be simulated by altering I_s , β cell dysfunction by reducing K_4 and/or K_5 and vascular defect slowing down transport of glucose and insulin to brain and other organs by altering K_8 , K_9 and K_1 . Mental stress is assumed to increase K_{10} . By differentially altering these parameters the model can separately and collectively examine the effects of insulin resistance, β cell dysfunction, reduced rates of diffusion across BBB and stress on the glucose tolerance curve.

5.5.2c T2DM- causal analysis

We ask the question which minimal set of changes can give rise to stable increase in fasting glucose, changes in the glucose tolerance curve characteristic of type 2 diabetes along with other patterns listed above. The putative causal factors are examined individually and in combination. The factors include insulin resistance (Decreased I_s), subnormal β cell response (Reduced K_4 and/or K_5), reduced insulin independent glucose uptake (decrease in K_1), reduced blood to brain transmission of glucose (K_8) and insulin (K_9) and stress related increase in brain glucose consumption (K_{10}). Subnormal or defective vasculature is expected to decrease K_1 , K_8 and K_9 proportionately, however the decrease in the three parameters may not be proportionate if the vasculature in different parts of the body is differentially affected. Also glucose transporters and their expressions can alter in tissue specific manner. Therefore we allow proportionate as well as differential decrease in the three parameters.



5.6 Results:

5.6.1 Steady state solutions:

We observed that the steady state solutions and the autonomic iteration simulations match well in the end result except when the limits of L and K_4 are reached. However, it took a long time to reach the desired level by iterative approach, particularly when the desired level was substantially different from the starting level. In real life major changes in vascular function or glucose transporter levels reflected in K_8 , K_9 , K_{11} , happen gradually. Therefore the desired level can be attained by autonomic fine tuning over time. Since in the iterative simulations, a long time was required to reach SS, we used the steady state solutions for quicker results.

It can be seen that the steady state levels of PG in the model is a stable steady state since substituting $PG < \underline{PG}$ leads to a positive value and $PG > \underline{PG}$ leads to a negative value of $\frac{dPG}{dt}$. The same applies to plasma insulin, brain glucose and brain insulin. This ensures a stable steady state of glucose-insulin in the plasma as well as brain on fasting. From Eq.5, Eq.6, Eq.7 and Eq.8 we see that, FG does not change by altering I_s or FI . This is compatible with the empirical findings that insulin receptor knockouts and insulin suppression experiments fail to increase fasting plasma glucose. This result is unique to our model and is due to segregating fasting and post meal glucose regulation mechanisms by using thresholds. This is the only explanation offered so far, for patterns observed in anomalies 9, 16 and 17.

The steady state solution of our model shows that FG can increase as a result of increase in K_{10} or decrease in K_8 . That is if the glucose consumption in brain increases and/or the rate of glucose transport from blood to brain decreases. Both the effects are interdependent and the shape of the glucose response is decided by the interaction between K_8 and K_{10} . Increased K_{10} increases FG marginally and almost linearly when K_8 is large. When K_8 decreases even a marginal rise in K_{10} can induce disproportionately greater rise in FG (Figure 1). This means that stress induced hyperglycemia is unlikely to be seen in healthy individuals while it is more likely in individuals with reduced vascular transport, this explains anomaly 7.

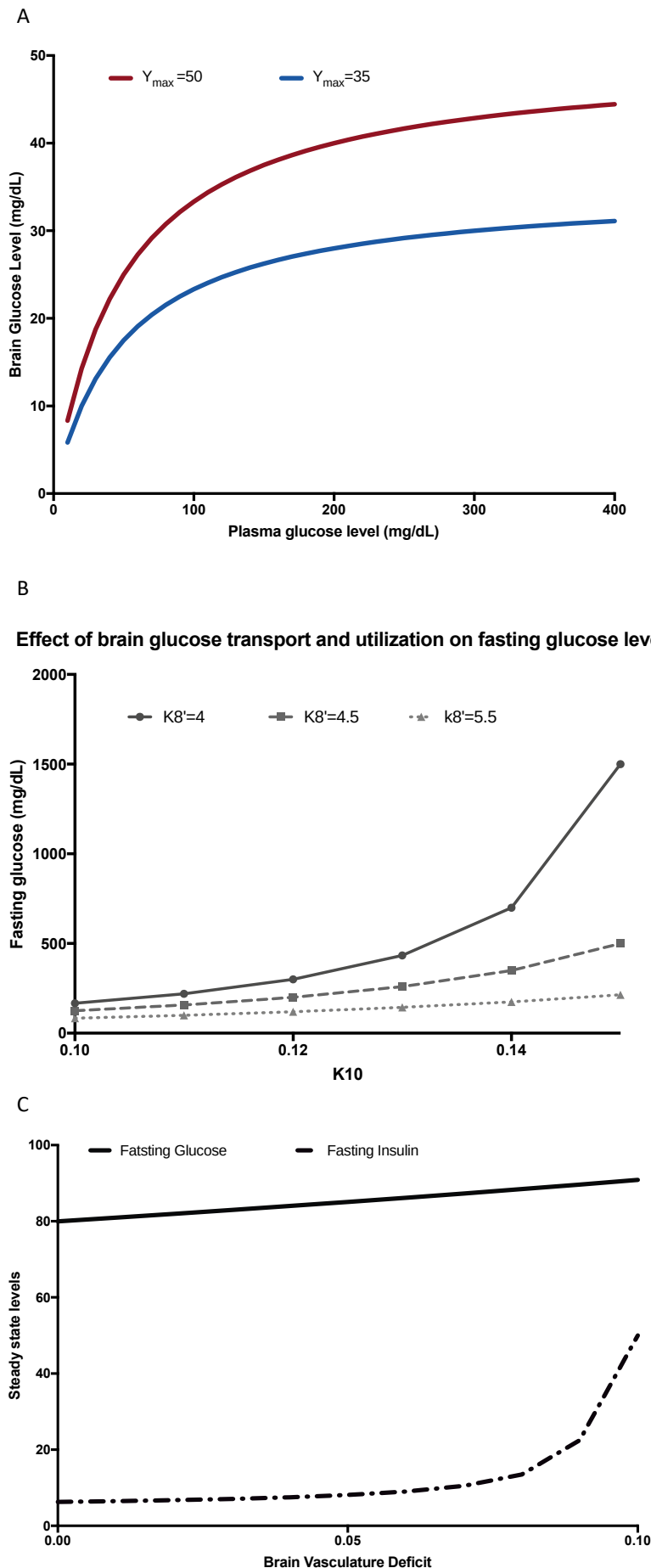


Figure 5.1: Steady state solutions

A. depicts a saturation relationship in glucose and insulin transport described by a Michaelis Menten type of equation has important consequences that can account for many phenomena observed in prediabetic and diabetic stages. For example, here for achieving an increase in Y by 6 units with a higher $Y_{\max}$, X needs to increase only by 6 units but at a lower $Y_{\max}$ for the same target increase in Y 25 units of increment in X is needed. This non-linearity explains many phenomena including prediabetic hyperinsulinemia and stress induced diabetes in our model of glucose homeostasis.

B. A simulation using the steady state model: It can be seen that as K_8 decreases, for the same change in brain requirement K_{10} , a non-linear escalated increase in plasma glucose is required

C. A simulation result assuming a correlated decrease in K_8 and K_9 . It is possible that while glucose shows a marginal increase, insulin increases substantially, since the parameters of glucose and insulin transport curves are different. This is a potential explanation for hyperinsulinemic normoglycemic state.



The origin of hyperinsulinemic normoglycemic condition can be explained by the difference in the parameters of the saturation curves of transport dynamics of glucose and insulin. If the insulin diffusion is assumed to be nearer to saturation and glucose diffusion is sufficiently away from saturation, at the same level of vascular function deficiency, insulin will increase disproportionately more than glucose (Figure 1). This is a possible cause of hyperinsulinemic normoglycemic state, and the reason why hyperinsulinemia precedes hyperglycemia which requires neither insulin resistance nor compensatory hyperinsulinemic response. This explains anomaly 3 and 4 respectively. When this happens, both HOMA-IR and HOMA β increase although there is no change in true insulin resistance and compensatory insulin response. This gives a false impression of insulin resistance as clinically defined.

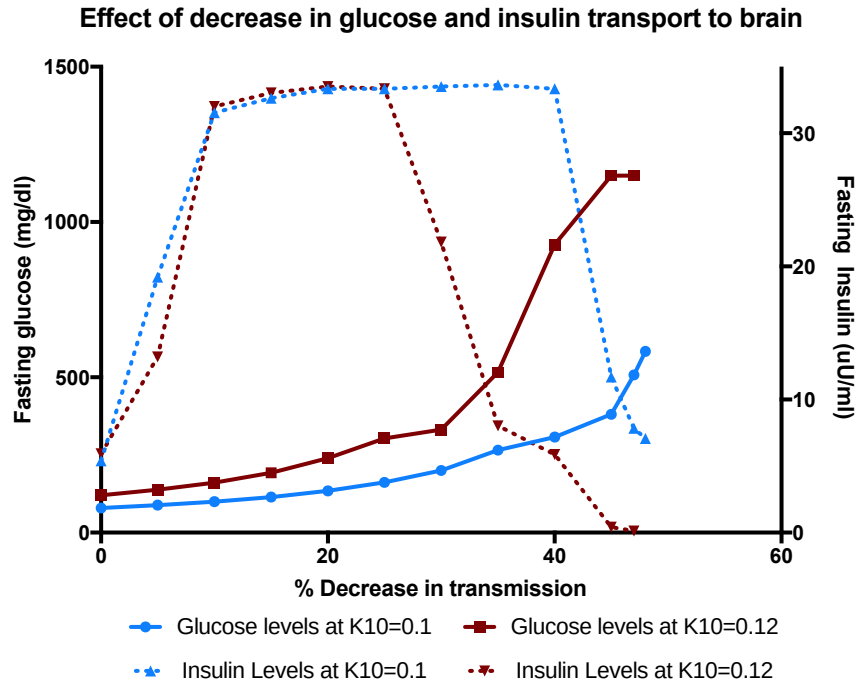


Figure 5.2: Effect of correlated decrease in K_8 and K_9 on fasting plasma glucose and insulin. Note that for a small to moderate decrease FI increases rapidly with marginal change in FG. However, after a threshold decrease in the transport rates FI declines sharply whereas FG increases rapidly. This pattern matches with the course of clinical T2DM without involving insulin resistance, compensatory hyperinsulinemia and β cell exhaustion. Simulation specific other parameters were $K_{1m} = 50$, $K_1 = 30$, $K_{2m} = 15$, $K_2 = 0.01$, $K_{5m} = 0.01$, $K_5 = 0.5$, $d_1 = 0.15$, $I_s = 1$, $BGt = 25$, $BIt = 5$, $K_{8m} = 80$, $K_8 = 5$, $K_{9m} = 0.3$, $K_9 = 0.8$, $K_{10} = 0.1$ (Blue line) and 0.12 (Red line).



Further as the transport saturation constant K_8 and K_9 continue to decrease proportionately, FG increases monotonically but FI shows a non-monotonic curve in which FI increases with moderate decrease in transport rates but decreases after a threshold decrease in transport rates. This non-monotonicity is predicted by the iterative autonomic inputs model. When the required L approaches or exceeds L_{max} , the steady state BG remains smaller than BG_t . The resultant increase in the sympathetic signal inhibits insulin release from β cells. This leads to reduced insulin secretion and insulin levels drop substantially. Thus, without bringing in additional factors the model explains the early phase rise in fasting insulin as well as the later phase decline in the course of T2DM (Figure 2).

This decline in the insulin response does not require β cell dysfunction as a causal mechanism but there are more complex possible effects on β cells. Simultaneous activation of sympathetic and parasympathetic inputs to β cells is implicated in β cell amyloidogenesis (Watve et al., 2014). Parasympathetic stimulation is known to increase β cell number. However, sympathetic signalling suppresses insulin release resulting in increased retention time of insulin along with amylin which beyond a threshold retardation may result in to spontaneous polymerization of amyloid protein leading to poisoning of β cells and amyloidogenesis (Watve et al., 2014). This is an alternative causal interpretation of β cell dysfunction and decline in its population. This mechanism has a built-in negative feedback loop giving rise to a steady state β cell population. This result is compatible with the finding in which the β cell population remains subnormal lifelong, explaining anomalies 11 and 12. This is in contrast with the dynamics expected by the classical thinking in which β cells are destroyed by gluco-lipotoxicity or oxidative stress. This mechanism has a built-in positive feedback cycle. As a part of the β cell population is destroyed, the insulin secretion is decreased which would increase glucose further accelerating gluco-lipotoxicity and oxidative stress and thereby β cell loss. Such a positive feedback vicious cycle can only stop with complete destruction of the β cell population. This prediction of the classical model does not match the finding of sustained presence of subnormal β cell population in T2DM.

By the classical model, prolonged sustained exercise in the fasting state should result into lower plasma glucose. However, experimental increase in glucose uptake resulting from physical activity during fasting does not necessarily result into a decrease in FG . This according to our model is mainly because as FG starts decreasing, BG decreases consequently. BG being lower than BG_t induces sympathetic mediated increase in L and the change in FG by physical activity



is more or less compensated. This may happen through the agency of glucagon as well, since a strong link between autonomic signals and glucagon secretion is known. Therefore, at times plasma glucose may actually increase after intensive exercise. Further, if exercise involves heightened activation of cerebellar mechanisms of coordination, K_{10} may also increase resulting into higher rather than lower FG in response to exercise, explaining anomaly 8.

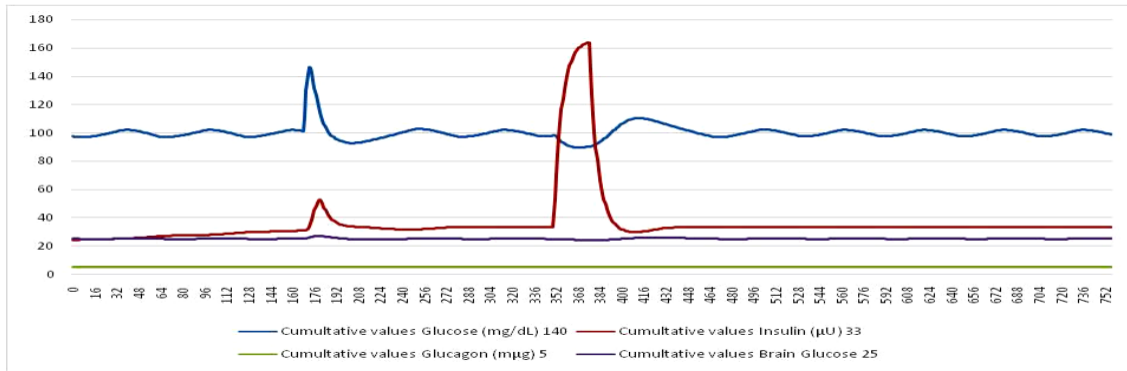
A high dose of exogenous insulin results into increased plasma glucose after a time lag and is known as Somogyi phenomenon. The phenomenon is conditional and not seen in every diabetic. It is said to involve the counter-regulatory response on facing central hypoglycemia. However, often this response is seen without peripheral hypoglycemia. This is best explained by reduced K_8 that gives rise to decreases BG/FG ratio. With smaller K_8 , at apparently normal or increased plasma glucose, brain glucose can still be lower than the target which gives a sympathetic signal to increase L . Simulations using the autonomic iteration model shows this phenomenon quite well at low K_8 . At the same level of insulin infusion, at lower K_8 there is more intense hyperglycemic response (Figure 3). This would explain the anomaly 14. A model without accommodating changes in K_8 does not predict Somogyi phenomenon without an obvious hypoglycemic state.

The response is restricted to a narrow set of conditions. For intense hyperglycemic response to insulin administration, it is necessary that insulin sensitivity is good, BG is close to BG_t before insulin transfusion, and K_8 is subnormal.



Potentially one can visualize two distinct possible classes of reasons why blood sugar increases in response to brain injury. In the first, there is some impairment in the glucose regulation mechanism. In the second, the requirement for glucose is increased during the wound healing process and hyperglycemia is a mechanism of the body to meet the demand. It is known that infections such as meningitis, stroke or haemorrhage leads to transient hyperglycemia. Such hyperglycemia is often accompanied by lower *BG* levels (Schut et al., 2009; van Veen et al., 2016). In such cases, hypoglycemic treatments can be counterproductive and result into delayed or derailed repair process. This is a likely reason why in patients under intensive care, strict regulation of plasma glucose increased mortality instead of the expected decrease, explaining anomaly 15 (The NICE-SUGAR Study Investigators, 2009).

A. At 90% transportation rate across BBB



B. At 70% transportation rate across BBB

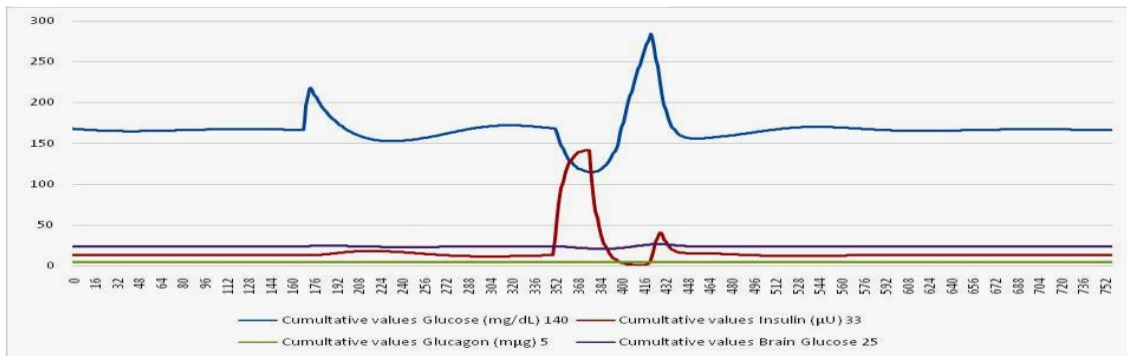


Figure 5.3: Effect of high dose insulin on peripheral glucose under two rates of glucose and insulin transport across the blood brain barrier (Correlated decrease in K_8 and K_9). A paradoxical rise in blood sugar following insulin administration is more prominent when the transport rate is substantially lower than normal. Other parameters ($K_{1m}=50$, $K_1=30$, $K_{2m}=15$, $K_2=0.08$, $K_{5m}=0.01$, $K_5=15$, $d_1=0.15$, $I_s=1$, $BG_t=25$, $BI_t=4.5$, $K_{8m}=80$, $K_8=5$, $K_{9m}=0.3$, $K_9=0.8$, $K_{10}=0.1$)



When hyperglycemia is a process of meeting increased glucose demand or compensating subnormal transport, removal of plasma glucose by any means is expected to increase L . This is observed when SGLT2 inhibitors decrease the urinary threshold and drive out plasma glucose. If increasing L is not sufficient to restore the required glucose level, there is a shift to ketogenesis since the brain can use keto acids as alternative source of energy. Therefore, increased L with or without increased ketogenesis is expected following SGLT2 inhibitors. This explains anomaly 13.

5.6.2 Glucose tolerance curve

Unlike the steady state predictions, the patterns of post feeding glucose curve by our model are not qualitatively different from classical models. The area under the curve, height of the peak, time required to return to steady state and the time difference between glucose and insulin peaks are increased by decreased I_s , K_5 , or K_8 individually or in combination. Decrease in I_s or K_5 does not increase FG but changes the shape of the glucose tolerance curve. Decreased K_8 may alter both simultaneously. The altered curve shows the three typical features namely taller peak, delayed return to steady state and longer gap between glucose peak and insulin peak. This result is not unique to our model and classical models also show the three features. The classical model results into simultaneous and proportionate alterations in the fasting as well as post meal glucose and therefore fails to explain the NFG-IGT state. In our model, decreasing I_s or K_1 without a change in K_8 results into an NFG-IGT state, explaining anomaly 5 and 6.

5.6.3 Population simulations

For the steady state as well as post glucose load dynamics we give population distributions to K_1 , K_5 , K_8 , K_9 , I_s and also incorporate normally distributed error in glucose and insulin measurements in fasting and post meal sampling. We also incorporate correlated changes in K_1 , K_8 and K_9 which are expected as a result of hypo-vascularization in the brain. These simulations are run to observe whether we get the anomalous correlations in fasting versus post meal state and in the HOMA indices as observed empirically (Chawla et al., 2018; Diwekar-Joshi & Watve, 2020).

By classical models the regression correlation parameters of glucose-insulin relationship are not different in fasting state versus post glucose load although the range of variables is different as shown previously by Diwekar-Joshi and Watve (2020). Also, if we assume HOMA-IR to



faithfully reflect insulin resistance and HOMA β to faithfully represent β cell response, then there is no reason why the two indices should be correlated.

The assumption behind our model that there are different mechanisms at work under fasting versus post glucose load condition is necessary to explain the large difference in the regression correlation parameters in fasting versus post meal levels. If the same set of mechanisms in the fasting and post meal conditions are operational, whatever the model used, it is imperative that fasting correlation regression parameters are stronger or comparable to post meal parameters. Simulations with our model are able to give poor *FG-FI* correlation along with strong post meal glucose insulin correlation under multiple conditions, explaining anomaly 16 (Figure 4). When the variance in K_8 and K_9 is small but that in K_1 , K_5 , G_t and I_s individually or in combination is large, the fasting correlations are weak and post meal correlations strong; the post meal glucose-insulin regression slope is substantially greater than *FG-FI* slope. Also, whenever *FG-FI* correlation is weak, the indices HOMA-IR and HOMA β are strongly correlated similar to the epidemiological data. The difference between fasting and post meal regression correlation patterns is not predicted by the classical models and is unique to our model which assumes different set of regulatory mechanisms in the fasting and post meal state, incorporating anomaly 17.

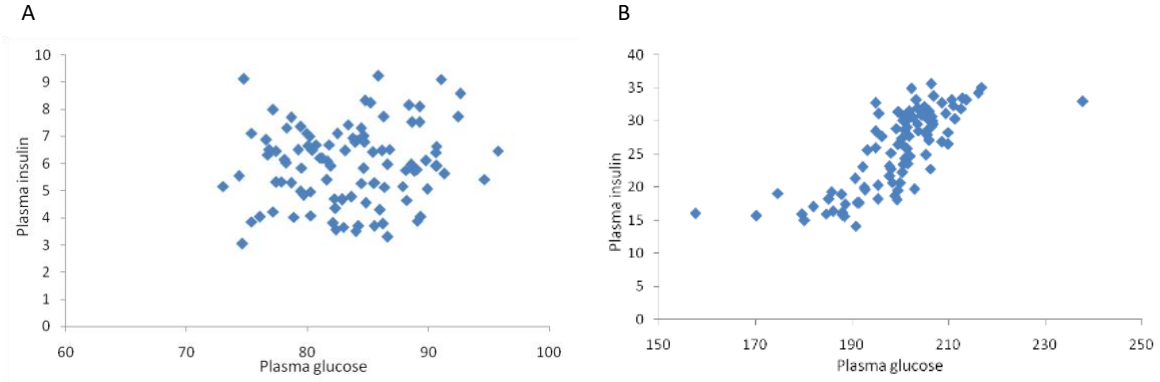


Figure 5.4: Plasma glucose and insulin correlation in the fasting steady state (A) and post meal (B) in simulations. The empirical finding that there is a strong positive correlation in the post meal data but poor correlation in fasting steady state was possible under a variety of conditions in our model. Depicted here the simulation results (post meal $R^2=0.57$, steady state $R^2=0.014$) in which only K_1 was given a wider population distribution. (mean (S.D.) $K_1 = 35(7)$, $K_5 = 10(0.00001)$, $K_8 = 5(0.000005)$, and $K_9 = 0.8(0.000008)$). Other parameters ($K_{1m} = 50$, $K_{2m} = 15$, $K_2 = 0.05$, $d_1 = 0.15$, $I_s = 1$, $BGt = 25$, $BI = 4.5$, $K_{8m} = 80$, $K_{10} = 0.1$).

5.6.4 Effect of glucose normalization on arresting diabetic complications and mortality

By classical thinking, chronic hyperglycemia is responsible for the diabetic complications and preventing hyperglycemia should arrest complications. In contrast the thought behind our model is that vascular problems are primary which alter the rate of glucose insulin transport to the brain and hyperglycemia is an offshoot symptom that may not be causal to diabetic complications. The diabetic complications can arise directly from the vasculopathy. Therefore, controlling glucose may not have any effect on diabetic complications as expressed in anomaly 18. On the other hand, forcefully reducing plasma sugar without addressing vascular problems can create more severe glucose deficiency in the brain and other organs, thereby turning counterproductive. Because of the saturating dynamics of transport, a curvilinear relationship is expected between plasma glucose and brain glucose in such a way that moderate reduction in hyperglycemia will change brain glucose availability marginally whereas tight glucose regulation can have disproportionately larger effect (Figure 5). Therefore, tight glucose regulation may increase mortality and other adverse outcomes. This prediction is compatible with some of the tight versus moderate control clinical trials including ACCORD, NICE sugar trial and UGDP.

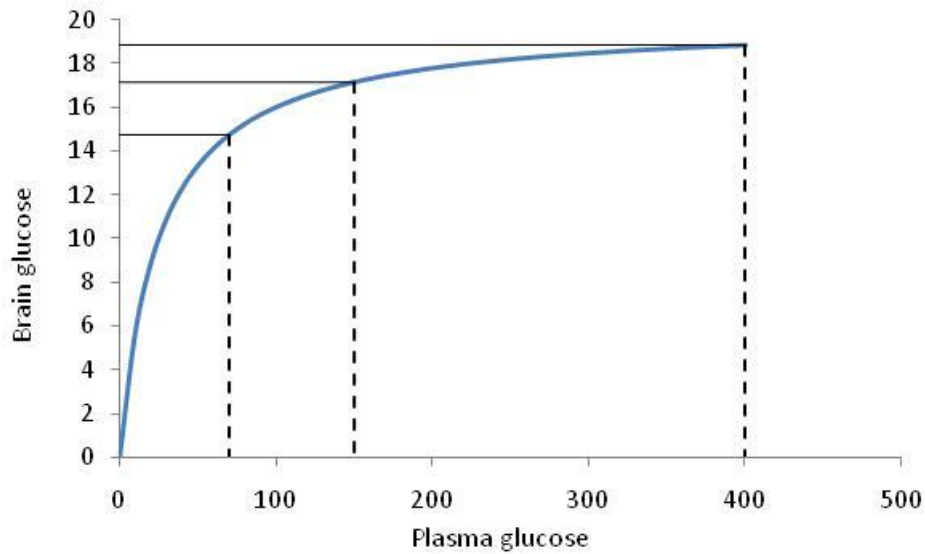


Figure 5.5: Effect of glucose lowering on brain glucose availability. For example, a reduction in FG from 400 mg/dl to 150 mg/dl corresponds to a decrease in BG by 1.68 mg/dl, but further reduction from 150 to 70 mg/dl reduces BG by 2.41 mg/dl. Therefore a moderate sugar control may not affect brain glucose supply drastically but tight sugar control is expected to affect it more seriously.

5.7 Conclusion

The main inference from our model, stated most conservatively, is that a brain centered model can potentially explain most of the anomalies faced by peripheral models and therefore needs greater attention (Figure 6). If supported well, by exploring its testable predictions suggested below and possibly more, it has a potential to bring in a radical change in the fundamental view as well as clinical practice of T2DM.

5.7.1 Testability of assumptions and additional predictions:

The assumption that the thresholds *PGT* and *PIT* can be modified by autonomous signalling needs to be tested empirically. Although currently the thresholds are known to be flexible, information about the conditions and mechanisms of change are poorly known. Our assumption that the parameters of saturation equation for glucose and insulin transport to the brain are different and under normal conditions insulin transport is closure to saturation than glucose transport can be tested with carefully worked out kinetics of the two transport mechanisms.

The assumption of our model that reduced transport of glucose and insulin to brain is the primary pathology of T2DM leading secondarily to hyperglycemia makes more predictions



that can be tested experimentally or epidemiologically. Experimentally specifically blocking GLUT1 transporters in the brain should lead to hyperglycemia. Conversely infusion of glucose directly to the brain should reduce peripheral hyperglycemia in the short run. This is already suggested by some experiments (Ono et al., 1983; Osundiji et al., 2012).

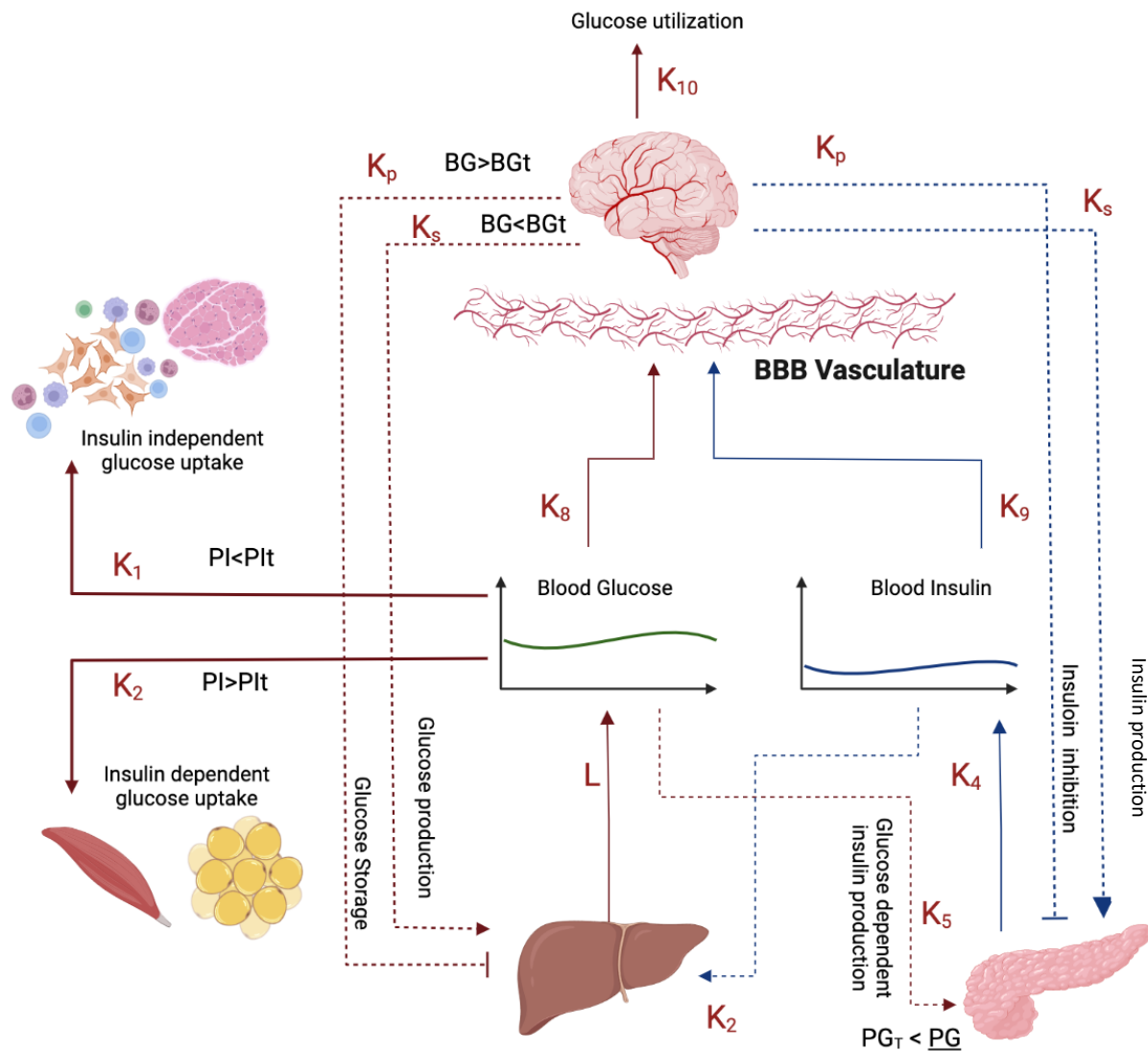


Figure 5.6: Effect of changes in vasculature in blood brain barrier. The model predicts that glucose and insulin transportation to the brain has proportionately major role in development of diabetic symptoms compared to other peripheral changes. The blood brain barrier vasculature hence may hold the key to understanding the shift from normal to diabetic condition. (created using BioRender)



It is also demonstrated that inducing primary endothelial dysfunction and reduced glucose transport to brain by endothelial deletion of hypoxia inducible factor HIF-1 α results in hyperglycemia (Huang et al., 2012). More careful research in this direction to reveal the cause effect relationship between vascular defects, brain glucose levels and plasma glucose levels will be enlightening. Epidemiologically hypoglycemia is shown to associate with dementia and other neuronal problems (Lipska & Montori, 2013; Meneilly & Tessier, 2016; Rhee, 2017; Yaffe, 2013), tight glycemic control led to higher mortality as compared to moderate control in many of the clinical trials (Klein, 2010; Schwartz & Meinert, 2004; The NICE-SUGAR Study Investigators, 2009) which demands investigations into the causal pathways. The question whether tight glycemic control leads to subtle neuronal changes in the long run as expected by our model needs careful investigation. The difference between fasting and post meal regression correlation parameters between glucose and insulin is an important epidemiological line of evidence we have used. Chawla et al (2017) and Diwekar-Joshi and Watve (2020) showed this pattern across four different data sets. The extent of this generalized pattern needs to be tested in multiple population data.

On the modelling front it is necessary to undertake comparative evaluation of the different models with respect to the battery of prediction that we listed here. Perhaps a few more predictions may be added. However, at present many of the models and their possible modifications are not explored sufficiently to see whether they can explain the currently unexplained patterns under certain set of conditions. A model prediction matrix would be an appropriate approach for such a comparative evaluation, but we may have to wait till all alternative models are explored sufficiently elaborately on which of the empirical patterns they predict, which ones they are compatible with and which ones they contradict. We have shown here that the brain centered model predicts or is compatible with all the patterns listed earlier and does not contradict any.

5.7.2 Possible causes of T2DM:

The classically believed causal factors namely insulin resistance and β cell dysfunction are not compatible with many of the empirical findings as shown by Diwekar-Joshi and Watve (2020). Another evidence of contradictions to the classical glucose homeostasis could be type 3 diabetes which is also a term used for hypothesis where Alzheimer's is proposed to be caused by insulin resistance and dysfunction of insulin-like growth factor in the brain. As discussed



earlier in the chapter, many diabetic patients face cognitive decline similar to Alzheimer's, with higher incidences of Alzheimer's in diabetic patients (Yang and Song 2013; Kandimalla, Thirumala, and Reddy 2017). Notably, insulin is found to be imperative for maintaining synaptic plasticity (Kandimalla, Thirumala, and Reddy 2017).

Much data now indicates that inadequate glucose supply to parts of the brain may underlie early stages of Alzheimer's. This is compatible with our hypothesis. Furthermore multiple pathophysiologies of Alzheimer's are directly linked to behavioral mismatches and a paper covering this work is under review right now (Watve and Keskar-Sardeshmukh, 2024, *under press*). So our hypothesis of behavioral mismatch and subnormal angiogenesis may explain Alzheimer's much better than the classical models.

In our model change in insulin resistance and β cell dysfunction were neither necessary nor sufficient to account for all the patterns. Nevertheless, they were helpful in accounting for the altered glucose tolerance curve, although other factors could also account for it independent of insulin resistance. Incorporating insulin resistance in our model and assuming it to work only in the post feeding state, patterns 1, 2, 5, 6, 9, 16 and 17 could be explained but not others. In short, our model does not rule out insulin resistance as a phenomenon, but implies that it may not be central to T2DM. Primary vasculature impairment reducing glucose and insulin transport in mutually correlated or uncorrelated manner could explain all patterns and therefore makes the most parsimonious causal hypothesis. Particularly, assuming that altered vascular function affects K_1 early followed by K_8 and K_9 is sufficient to explain all the patterns without alteration in I_s or any other factor. The apparent β cell dysfunction is an inevitable effect of higher degree of vascular dysfunction and therefore may not be needed as an independent causal factor.

Being open to alternative possibilities is an important virtue of science and it is particularly important with the limited clinical success of prevalent thinking along with mounting anomalous findings. Prevention of T2DM on a global scale has largely failed and treatment has limited and inconsistent success in arresting mortality and morbidities associated with T2DM (Brown et al., 2004; DeFronzo, 2010; Lee et al., 2021; Rosengren et al., 2008). Therefore, exploring alternative possible interpretations is a need of the time.

Triggered by multiple anomalies in the theory of glucose homeostasis and the origins of diabetic hyperglycemia, we have articulated here an alternative paradigm which potentially resolves in a logically and mathematically consistent manner many of the anomalous findings.



Being mathematically and logically sound and compatible with evidence is not a sufficient proof of a theory but certainly reflects on its potential to develop into a new alternative paradigm. For a large field such as T2DM multiple efforts would be needed to evaluate competing paradigms. We have suggested a few more testable predictions that can help in this task. There can be more possible ways of testing them which should come to light and used to reach robust conclusions which have the potential to change the clinical course of prevention as well as treatment of an important global health problem.

If our proposed causal inference that vascular dysfunction is primary to T2DM is supported by more careful investigations, it explains the failure of glucose normalizing treatment in arresting diabetic complications. Simultaneously it suggests alternative lines of treatments which should focus on normalizing vascular and neuronal function rather than focusing on glucose normalization. The deficiencies of stimuli normally required for growth factors and other angiogenic and neuroprotective factors can be forecasted as the best bet for the new approach. However, rigorous efforts are needed to strengthen the evidence base for selecting the right one amongst the alternative paradigms.



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CHAPTER 6

Into the unknown....(not really)



6.1 Conclusion

In my thesis and in earlier efforts in our lab, we have attempted to challenge and re-examine of the classical theory of diabetes by employing various approaches. The conventional glucose-insulin centric view, which relies solely on the biochemical effects of insulin and glucose metabolism to explain the development of insulin resistance, hyperglycemia, and hyperinsulinemia after weight gain, is challenged when we observe these phenomena in different species. We observe that the relationship between fat accumulation, insulin resistance, hyperinsulinemia, and hyperglycemia varies across species. Moreover, the development of insulin resistance and hyperglycemia does not necessarily lead to a diseased morbid condition; in some species, it even results in a longer lifespan.

We further analyse the evidence supporting the belief that hyperglycemia is responsible for diabetic complications and normalizing glucose can arrest them by studying large, institutionalized clinical trials that report the effects of intensive versus conventional glucose lowering. However, even after compiling decades of work, we do not find conclusive evidence that lowering blood glucose levels in diabetics can decrease the incidence of morbid complications.

I also conducted experiments in an STZ-induced mouse model to study the role of beta cell destruction in the development of type 2 diabetes. Although inconclusive, we found several indications that beta cell destruction is unlikely to be the only mechanism of STZ-induced diabetes in animals.

Finally, we developed a comprehensive model that incorporates all the existing caveats and anomalies in the study of type 2 diabetes, aiming to provide a meaningful understanding of glucose homeostasis in humans. The central theme of the model is the incorporation of the neuroendocrine axis, assuming that brain glucose and insulin levels are crucial to the system. This model successfully explains most of the existing anomalies in our understanding of type 2 diabetes and predicts that the disease may primarily be due to vascular changes, leading to decreased transportation rates across the blood-brain barrier and neuroendocrine reprogramming. This model accurately recapitulates the progression of the disease, leading us to believe that diabetes may be primarily caused by vascular pathologies. Vasculopathy is known to be central to diabetic complications, but the classical thinking has been that chronic hyperglycemia leads to vasculopathies. We suggest a reversal of the cause-effect relationship



where sedentary life style directly affects angiogenesis and other symptoms arise from the resulting vascular defects. This has revolutionary implications for T2DM prevention as well as treatment. However, the clinical picture is as yet resistant to change.

Is it surprising? Perhaps looking at history may help us here.

6.2 Will Lessons from history help us predict the future?

A major hallmark of a scientific theory is its contribution to increasing predictability of the system under study. Historical analysis, on the other hand, looks with hindsight at events in a critical and analytical framework but generally does not claim to predict the future. If the rules discovered by historical analysis help in predicting, the analysis can be said to have a scientific value. The celebrated 1962 book “The structure of scientific revolutions” by Thomas Kuhn was based on historical analysis of several revolutionary discoveries mainly in the history of physics and chemistry (Kuhn 1970). Kuhn’s explanation of progression of a scientific revolution was in itself revolutionary in understanding the progression of science towards truth. Using historical analysis of revolutionary concepts in fields of physics and chemistry Kuhn emphasizes on 4 stages of scientific progression: (i) Stage 1 is described as a time when no scientific consensus has been reached regarding the phenomena and there are conflicting theories with diverse explanation. (ii) Stage 2 involves a time when a paradigm (basic assumptions, principles and methods) has been established. Most of the scientific community now works towards detailing the paradigm. At this stage, most of the events which do not align with the accepted paradigm are counted as anomalies, and different tinkering in paradigm are made to accommodate these anomalies. (iv) Stage 3 begins when overtime anomalies accumulate such that tinkling the paradigm model now would challenge the basic assumptions. And this is when the crisis begins as resolving the anomaly would mean undermining the paradigm itself. (v) The final stage is marked by fall of the existing paradigm to give way to a new one, which now better explains the existing observations. Using the above as guiding principle, we would like to examine the field of type 2 diabetes mellitus (T2DM), to understand if a new paradigm is the need of the hour. Kuhn himself does not claim his analysis has a predictive potential, but we argue below that it has. The signs and symptoms described by Kuhn of a crisis situation are clearly visible in the field of type 2 diabetes research. Based on this it can be predicted with confidence that a major paradigm shift is due in this field.



The classical paradigm of type 2 diabetes comprises a series of 6 hypothesized phenomena in a cause effect chain (Watve 2013). (i) A positive energy balance leads to obesity (ii) Obesity leads to insulin resistance (iii) Insulin resistance is compensated by compensatory hyperinsulinemia (iv) at some stage the compensation fails presumably because of exhaustion of insulin producing beta cells and the blood sugar rises (vi) chronically increased blood sugar leads to a variety of diabetic complications (Ye 2013; DeFronzo et al. 2015). Based on this paradigm obesity prevention has been the major target of prevention and glucose normalization that of the treatment of type 2 diabetes. Although the picture looks very simple, logical and convincing; a number of ‘anomalies’ have accumulated in this field over the last two decades.

Anomalies play a crucial role in Kuhn’s synthesis, but the definition of anomalies remains elusive. Not all empirical findings that are not explained by the existing paradigm make ‘anomalies’ serious enough to give rise to a crisis. We need to classify the unexplained findings into two types. The ones which are not explained by the current paradigm but an extension of it possibly accounts for them. Second are the ones which directly contradict the paradigm. The latter is more likely to lead to a crisis than the former, although some subjectivity still remains with respect to what is perceived as ‘contradicting’.

Here we will highlight only a few selected serious anomalies faced by the field, while in reality there are more. (i) Although insulin is perceived as the agent responsible for regulating fasting steady state glucose, insulin receptor knockouts, insulin suppression and enhancement experiments unanimously failed to increase steady state plasma glucose levels (Brüning et al. 1998; Blüher et al. 2002; Kadowaki 2000; Costes and Butler 2014; Diwekar-Joshi and Watve 2020). (ii) Contrary to the compensatory insulin response hypothesis there is mounting evidence that hyperinsulinemia precedes insulin resistance (Gray et al. 2010; Abdul-Ghani and DeFronzo 2021; Corkey and Shirihai 2012; Pories and Dohm 2012; Shanik et al. 2008). If hyperinsulinemia is not a compensatory response to insulin resistance, the failure of compensation explanation become redundant. (iii) Insulin resistance itself is a circular and thereby unfalsifiable concept (Shinde, Ranade, and Watve 2021) that in Kuhn’s words “no experimental work could by itself have upset.” (iv) The cause-effect relationship between obesity, insulin resistance and hyperglycemia are chaotic. Contrary to the classical paradigm hyperinsulinemia is said to be the cause of obesity (Ludwig and Ebbeling 2018), or insulin resistance the cause of obesity (Lustig 2008). Not only the cause-effect relationship is confused, even correlation is weak, the median variance in insulin resistance explained by obesity being



only around 15% (Vidwans and Watve 2017). Bariatric surgeries, claimed to be the sole effective means of reversing T2DM, does not work by weight loss as perceived earlier. In fact, better glycemic control and insulin sensitization happens immediately after bariatric surgery although weight loss is delayed (Sugerman 2003; Schauer et al. 2003; Leichman et al. 2008; Rubino et al. 2010). On long term follow up, in 20% patients diabetes reappears but without regaining weight (Sjöström 2013). (v) The causes of beta cell dysfunction are paradoxical, circular and incompatible with mathematics. It is postulated that obesity causes desensitization of beta cells via oxidative stress due to excess of glucose, leading to lowering of insulin secretion due to beta cell dysfunction. This further becomes the primary cause of hyperglycemia. But in actuality type 2 diabetes exhibits hyperinsulinemia, so to now fit this diametrically opposite observation, it is hypothesized that after beta cell dysfunction, the healthy beta cells try to over-compensate and hence leading to hyperinsulinemia (Cerf 2013). (vi) sugar normalization is unable to prevent diabetic complications and any positive effects observed of drug administration are independent of sugar. Sugar normalization clinical trials have shown that intensive lowering of glucose from disease state levels to normal levels in T2DM patients in fact has detrimental effects on survival (Ismail-Beigi et al. 2010).

In spite of the numerous anomalies, most researchers in the fields do not appear to have expressed the need for a radical revision of the theory. This is not a surprise since Kuhn predicted this response quite well.

“.....even a discrepancy unaccountably larger than that experienced in other applications of the theory need not draw any very profound response.”

“Persistent and recognized anomaly does not always induce crisis.”

Instead of an immediate need to rethink, there is a proliferation of context specific explanations that attempt to give an “explainawaytion”. In Kuhn’s words, “proliferation of versions of a theory is a very usual symptom of crisis.” There is attempt to “push the rules of normal science harder than ever to see, in the area of difficulty, just where and how far they can be made to work.” “They will devise numerous articulations and ad hoc modifications of their theory in order to eliminate any apparent conflict”. The pushed explanations are not sound because “a discrepancy corrected in one place (is) likely to show up in another.” This pattern can be seen beautifully in explaining how obesity leads to insulin resistance, or how chronic hyperglycemia causes diabetic complications. For both, there is abundance of hypotheses with many internal



contradictions. Some of the hypotheses have been falsified by experiments but they continue to be used as per convenience(Watve 2013)This reflects the rigidity of science as described by Kuhn. “They do not renounce the paradigm that has led them into crisis. They do not, that is, treat anomalies as counter-instances, though in the vocabulary of philosophy of science that is what they are.” “Scientists fail to reject paradigms when faced with anomalies or counterinstances. They could not do so and still remain scientists.” Possibly the only place where Kuhn’s prediction hasn’t turned out to be true is “anomaly may evoke crisis if the applications that it inhibits have a particular practical importance.” In spite of having obvious application for T2DM treatment, and the stark failure of treatment in curing diabetes, the crisis is surprisingly not recognized by most people in the field.

All the examples used by Kuhn are from physics and chemistry. Biology handles orders of magnitude complex systems and cause effect relations are much more complex in biology. In addition, the culture and mindsets of researchers in biology is also likely to be substantially different than that of physicists. Therefore, the process of anomalies leading to crisis, and the response of researchers to crisis in biology might be more complex than that in physics. We can perceive some biology specific factors not described by Kuhn. Biology is inherently complex but often the complexity is used as an excuse to ignore logical contradictions. Unlike physics where problems have mostly quantifiable results, complex biological problems rely majorly on empirically derived results which are mostly qualitative in nature. This is particularly true about mathematical inconsistencies which are considered a serious lapse in theory by physicists but biologists often tolerate them. For example, a hyperinsulinemic normoglycemic prediabetic state cannot be obtained in a steady state solution of glucose homeostasis models (Watve and Ojha 2022). But this is rarely discussed or tried to be resolved.

The research in the field is getting increasingly complex using high tech tools including genomics, epigenomics, transcriptomics, proteomics and metabolomics. Although a large number of genetic variants are identified by GWAS studies their combined variance explained is small, which is known as missing heritability (Manolio et al. 2009; Loh et al. 2022; Drong, Lindgren, and McCarthy 2012; Stančáková and Laakso 2016). Other tools have added large amount of data of board but the anomalies remain unexplained. Most of the current research in the field follows Galisonian or Dysonian (Quirós and Villa 2008; Dyson 2012; Jogalekar Ashutosh, n.d.) , i.e. tool driven revolution, rather than Kuhnian or ideas driven revolution.



The accumulating anomalies in the field of T2DM, indicate to a clear rise of a crisis state in need of paradigm shift. There could be additional possible reasons for the slow transition from “anomalies to crisis to a new paradigm” in field of T2DM like (i) When Thomas Kuhn wrote about the nature of scientific revolutions, peer reviews were not a universal practice. They became a mandatory norm by the 1970s. Peers reviews often add to the resistance to major conceptual novelties (Watve 2017; Milind Watve 2019). Therefore, publishing experimental results and interpretations incompatible with existing paradigm becomes even more difficult. (ii) In the field of biomedicine, the pharma industry has a strong influence on the direction as well as funding of research and research that makes the ground more difficult to drug discovery has a smaller chance of getting support. (iii) With increasing specialization, the interacting communities become increasingly narrower and seniorities, hierarchies and xenophobia becomes stronger (Vidwans and Watve 2017). According to Kuhn, “Almost always the men who achieve these fundamental inventions of a new paradigm have been either very young or very new to the field whose paradigm they change.” Greater the specialization of a branch, more difficult it is to accept an outsider and his/her views.

Perhaps the most important trap is the hen and egg problem of research. “.... a scientific theory is declared invalid only if an alternate candidate is available to take its place.” “To reject one paradigm without simultaneously substituting another is to reject science itself.” In funding intensive fields, a new paradigm cannot arise with sufficient strength and evidence base unless it receives the required funding support. Funding for a novel concept is unlikely until the existing paradigm is rejected or perceived as inadequate. For this to happen an alternative paradigm should be perceived. The only way out would be to pay sufficient attention to the existing anomalies, admit the inadequacies of the classical paradigm of T2DM and be critical but open for alternative ideas.

In a field like T2DM, the delay in recognizing anomalies and feeling the crisis may have been responsible for the suffering of an entire generation or more of diabetics. It is necessary now for the diabetes research community to have a critical look at all the evidence, address the anomalies sincerely, make the research environment conducive to arising alternative paradigms and then carefully chose among the emerging new ones.

If this happens, it would be a historical example of how the study of history of science can predict and further the progress of science. In future we should aim at crystallizing with greater



clarity the diagnostic relevance of anomalies, the signs and symptoms of crisis and its eventual culmination into one of the three possible outcomes of crisis. In Kuhn's words, "And all crises close in one of three ways. Sometimes normal science ultimately proves able to handle the crisis-provoking problem despite the despair of those who have seen it as the end of an existing paradigm. On other occasions the problem resists even apparently radical new approaches. Then scientists may conclude that no solution will be forthcoming in the present state of their field. The problem is labelled and set aside for a future generation with more developed tools. Or, finally, the case that will most concern us here, a crisis may end with the emergence of a new candidate for paradigm and with the ensuing battle over its acceptance."

Let us end with a wish for the third outcome that gives rise to effective measures of prevention and treatment leading to near elimination of T2DM from the human population.



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Epilogue

(Personal)

Thank you reaching the end of this thesis. I am truly grateful that you had the perseverance to endure through this arduous journey. This is completely unfiltered and unrevised form of gratitude and I hope I get to keep it as it is until the end. I would like to use this space now to heartfully thank everyone that I have already thanked in the acknowledgement. I just wanted everyone who reaches to the end of this thesis know, that I have been extremely lucky to be surrounded by a wonderful set of people both professionally and personally. Even when the situation was difficult or even when these same people were difficult, they only got me to be better. And at the end made me believe in myself. For every (any) student reading this, trust me you are not supposed to do any of anything alone. I wouldn't have been able to complete 10% of this work if I didn't learn to ask for help. And at the end I would just like to quote one of my favourite's "Happiness can be found, even in the darkest of times, if one only remembers to turn on the light". Everyone I have met in this journey has been this switch to light. Thank you!!