Association of SIRT6 with obesity and diabetes

By

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Declaration

It is hereby declared that

1. The thesis submitted is my original work while completing my degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

Sameha Tabassum

Approval

The project titled "Association of SIRT6 with obesity and diabetes" submitted by Sameha Tabassum (18346039) in spring 2022 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on November 2022.

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Ethics Statement

The study does not involve any kind of animal and human trial.

Abstract:

SIRT6 is one of the sirtuin family members, a kind of NAD+-dependent histone deacetylase and ADP-ribose transferase enzyme. It controls aging, cancer, obesity, insulin resistance, inflammation, and energy metabolism, playing a significant role in both physiological and pathological processes. Sirt6 levels and function diminish with age and overnutrition, two key risk factors for obesity and diabetes, which results in improper glucose and lipid metabolism. Mice with SIRT6 ablation across the body have acute hypoglycemia. SIRT6 deficiency enhances diet-induced obesity and insulin resistance and causes liver steatosis. Diabetes and obesity are protected against SIRT6.This review examines the data supporting SIRT6's emerging function as a regulator of metabolism in mammals and lists its key roles in obesity and diabetes.

Keywords: SIRT6, obesity, type 2 diabetes, gluconeogenesis.

Dedication

I dedicate this work to everyone who inspired me in my work and especially to my supervisor.

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List of Acronyms

NAD - Nicotinamide adenine dinucleotide

T1DM- Type 1 diabetes mellitus

IDDM- Insulin-dependent diabetic Mellitus

DKA- Diabetic ketoacidosis

GADA- Glutamic acid decarboxylase autoantibodies

ICA- Islet cell autoantibodies

IAAs- Insulin autoantibodies

ZnT8- Zinc transporter isoform 8

IgG- Immunoglobulin G

T2DM- Type 2 diabetes mellitus

LADA- Latent autoimmune disease in Adults

NIDDM- Non–insulin-dependent diabetes mellitus

GDM-Gestational Diabetes Mellitus

SGLT2- Sodium-glucose cotransporter-2

T2D-Type 2 diabetes

HDL- High-density lipoprotein

BMI- Body mass index

GLP-1-Glucagon-like peptide 1

DIO- Diet-Induced Obesity

GIP- Gastric inhibitory polypeptide

FDA- Food and Drug Administration

LDH- Lactic acid dehydrogenase

PDK1- Pyruvate dehydrogenase kinase 1

PDK4- Pyruvate dehydrogenase kinase 4

GLUT1-Glucose transporter 1

TPI- Triose-phosphate isomerase

IGF-1-Insulin-like growth factor-1

KIF5C- Kinesin family member 5C

HFD-High-fat diet

PPARs- Peroxisome proliferator-activated receptors

DGAT1-Diglyceride acyltransferase 1

ATGL- Adipose triglyceride lipase

Pepck- Phosphoenolpyruvate carboxy kinase

PGC- Lalpha-proliferator-activated receptor gamma-coactivator 1

FoxO1-Forkhead box protein O1

SREBF1-Sterol regulatory element-binding transcription factor 1

SREBF2-Sterol regulatory element-binding transcription factor 2

SNPs- Single nucleotide polymorphisms

CPS1- Carbamoyl-phosphate synthase 1

Chapter 1

1. Introduction

1.1. An overview of SIRT6 in diabetics and obesity

Sirtuins are a highly conserved member of NAD+-dependent deacetylases and ADP-ribosyl transferases that perform critical regulatory functions in physiological and pathological processes in organisms. They control aging, longevity, cancer, obesity, insulin resistance, inflammatory reactions, and energy anabolism (Kuang et al., 2018). SIRT2, the first member of the sirtuin family, was first identified in Saccharomyces cerevisiae. Seven sirtuins (Sirt1-7) have now been discovered in mammals, each containing a conserved sirtuin core domain that confers NAD+-dependent deacetylase activity (Kuang et al., 2018).

Every single member contains a different subcellular location, goal as well as performance. SIRT1 and Sirt2 were discovered in the nucleus and cytoplasm. Sirts 3, 4, and 5 are present in mitochondria, while SIRTs 6 and 7 are found in the nucleus. Sirt1-3 exhibits strong deacetylase activity, while Sirt4 exhibits ADP-ribosyl transferase activity (Theos et al., 2005). Sirt5 has weak NAD+-dependent deacetylase, deacetylase, and deadenylase activities. Sirt7 has recently been discovered to be a highly selective deacetylase. Sirt6 possesses a variety of enzymatic activities, which include NAD+-dependent acetyl and long-chain acyl group deacetylase activity and mono ADP-ribosyl transferase activity. The best-studied sirtuin is Sirt1, and research on Sirt6 is simply beginning (Kuang et al., 2018).

Sirt6, a NAD+-dependent deacetylase, was first cloned from a human spleen cDNA library. Recent studies suggest, decreased Sirt6 activity is associated with obesity and diabetes. Aging and overeating

Table 1: Sirtuins with their location and function

Sirtuins	Locations	Functions	Reference
SIRT1	Cell nucleus	Metabolism, inflammation	(Carafa et al., 2016)
SIRT2	Mainly cytoplasm	Cell cycle	(Carafa et al., 2016)
SIRT3	Mitochondrial compartment	Metabolism	(Carafa et al., 2016)
SIRT4	Mitochondrial compartment;	Insulin secretion	(Carafa et al., 2016)
SIRT5	Mitochondrial compartment;	Unknown	(Carafa et al., 2016)
SIRT6	Cell nucleus	DNA repair	(Carafa et al., 2016)
SIRT7	Cell nucleus	Recombinant DNA transcription	(Carafa et al., 2016)

are two key risk factors for obesity and diabetes, both of which result in diminished Sirt6 levels and function, resulting in aberrant glucose and lipid metabolism. Sirt6 systemic ablation in mice resulted in severe hypoglycemia (Kuang et al., 2018) . Sirt6 ablation in the liver promoted fatty liver. Sirt6 deletion in adipose tissue raised blood glucose levels and fatty liver, as well as diet-related obesity and insulin resistance. Sirt6 deletion in mice increased diet-related obesity and insulin resistance. Moreover, Sirt6 overexpression covered diet-related weight issues and insulin resistance (Morigi et al., 2018). Sirt6 appears to be important in lipid and glucose metabolism, according to current research. This review examines Sirt6's function in obesity and diabetes.

1.2. Pathophysiology of diabetes and obesity

1.2.1. Diabetes

1.2.1.1. Type 1 diabetes mellitus

T1DM, (type 1 diabetes mellitus) also known as type 1A DM or insulin-dependent diabetic Mellitus (IDDM) or juvenile diabetes, accounts for around 5-10% of all diabetes cases. It is a condition marked by T-cell-mediated death of pancreatic -cells, leading in insulin insufficiency and, eventually, hyperglycemia.

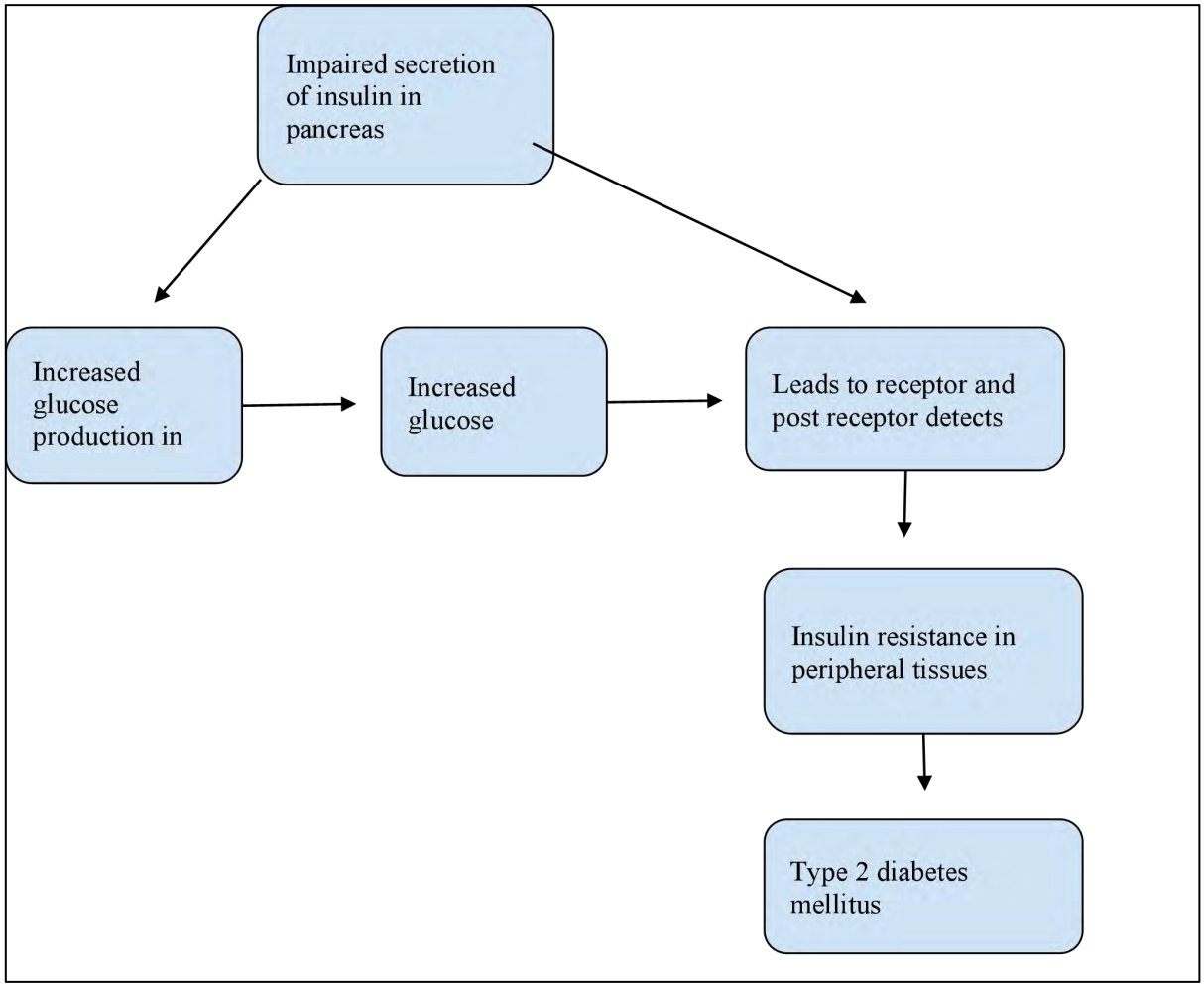

Figure 1: Pathophysiology of Type 1 diabetes mellitus

Pancreas Variability in the rate at which immune-mediated destruction of β-cells occurs often defines the ultimate progression of the disease. In some cases, in children and adolescents, there is abrupt β-cell destruction and subsequent failure, which can lead to diabetic ketoacidosis (DKA), which is often described as the first symptom of the disease (Banday et al., 2020). In others, the course of the disease is prolonged, and hunger leads to severe hyperglycemia, with or without ketoacidosis, only in the presence of physiological stress, such as severe infections or the development of other diseases. In some other cases, including adults, β-cells function to some extent to secrete just enough insulin to prevent ketoacidosis for many years (Meuleneire, 2008). However,

progressive insulin deficiency makes these individuals insulin-dependent, with severe hyperglycemia and subsequent development of ketoacidosis. Despite the variable course of this form of diabetes, patients develop severe or absolute insulin deficiency early, middle, or even late in life and depend on insulin treatment for survival. or absolute insulin deficiency, indicated by low or undetectable plasma C-peptide levels at any age, regardless of its onset.

These autoantibodies are associated with the immune-mediated β-cell destruction that is characteristic of this disease. These autoantibodies are linked to the immune-mediated cell death that is a defining feature of this illness. Glutamic acid decarboxylase autoantibodies (GADAs) like GAD65, islet cell autoantibodies (ICAs) to -cell cytoplasmic proteins like autoantibodies to islet cell antigen 512 (ICA512), tyrosine phosphatases autoantibodies IA-2 and IA-2, insulin autoantibodies (IAAs), and autoantibodies to islet-specific zinc transporter isoform 8 are among the auto (ZnT8) (Banday et al., 2020). A minimum of one amongst these autoantibodies are often used for clinical diagnosis of the disease, but typically many of those immune markers are observed in approximately 85-90% of her newly-onset T1DM patients. Among these autoantibodies, GAD65 is that the most vital, present in approximately 80% of all T1DM patients at diagnosis, followed by ICA in 69–90%, and everyone 54 Followed by IA-2α, present in -75%. - T1DM individual at the time of clinical presentation.

The IAAs are essential immune markers found in babies and younger kids who are prone to diabetes and occurrence decreases because the age of onset of diabetes increases. The presence of IAAs in those people who've now no longer been formerly handled with insulin is an essential indication of growing T1DM. IAAs are found in approximately 70% of all babies and younger kids at the time of analysis (Meuleneire, 2008). The IAAs additionally play an essential inhibitory position towards insulin characteristics in sufferers of insulin therapy. Although now no longer

frequently clinically massive however nevertheless, this immune reaction has been discovered with various ranges of severity in at least 40% of sufferers of insulin remedy and consequently suggests differential scientific manifestations (Banday et al., 2020). These autoantibodies in the main include polyclonal immunoglobulin G (IgG) antibodies and fluctuate their affinities and binding capacities towards insulin. IAAs can both be excessive insulin affinity/low insulin-binding potential or low insulin affinity/excessive insulin-binding potential. The low insulin affinity/excessive insulin-binding potential IAAs are answerable for scientific manifestations. At excessive titers, the binding of those antibodies to insulin prevents or delays its motion and is answerable for function hyperglycemia withinside on the spot postprandial period, which leads to noticeably multiplied insulin necessities observed through unpredictable hypoglycemic episodes (postprandial hypoglycemia) discovered later (Banday et al., 2020)

These autoantibodies expect extra scientific and diagnostic significance in a few instances, especially in adults, with late-onset of this sickness wherein the destruction of the pancreatic β-cells happens at a completely gradual price and frequently the sickness masquerades as in T2DM (Banday et al., 2020). In such instances, those autoantibodies permit the precise analysis of this ailment because the T1DM, in place of the maximum not unusual place T2DM. This sort of diabetes is usually defined as “Latent autoimmune disease in Adults (LADA),” additionally acknowledged as “slowly progressing insulin-based diabetes (Meuleneire, 2008).

LADA is the maximum not unusual place shape of grownup-onset autoimmune diabetes and debts for 2–12% of all diabetic instances withinside the grownup population of the autoantibodies, GADAs are the maximum essential and touchy markers for LADA observed through ICAs. However, the IAAs, autoantibodies to the tyrosine phosphatases—IA-2 and IA-2α, and autoantibodies to islet-unique zinc transporter isoform 8 (ZnT8) might be discovered in sufferers

with juvenile- or younger-onset T1DM are detectable in handiest a small range of instances in LADA (Banday et al., 2020). In a have a look at LADA, GADAs had been the handiest diabetes-unique autoantibodies detected in 68.6% of general screened topics while IA-2α and ZnT8A represented the single-kind autoantibody detections in 5% and 2.3% of all of the screened have a look at topics. In the identical look, multiple kinds of autoantibody become detected in 24.1% of having a look at topics. LADA is likewise every so often known as T2DM with ICAs.

1.2.1.2. Idiopathic diabetes

Idiopathic diabetes, consists of the varieties of diabetes which may be very similar to T1DM in presentation however characterized with the help of using variable nonimmune β-molecular disorder with no determined HLA affiliation in contrast to T1 diabetes mellitus and hence, occasionally it is defined as a different variety of DM. This kind of diabetes is well-known for having a strong genetic pattern and has only been identified in a small number of people of Asian or African-Caribbean heritage. The cause of idiopathic diabetes is still mostly unknown (Banday et al., 2020).

The disorder is characterized by the aid of using excessive, however various levels of insulin deficiency (insulinogenic) that may showcase episodic styles concomitant with various levels of severity and episodic DKA. These patients, therefore, can even additionally require an insulin substitute remedy first of all however the want for the remedy won't be absolute and might range in line with the episodic varieties of insulinogenic and ketoacidosis function of these forms of T1DM (Meuleneire, 2008).

1.2.1.3. Type 2 diabetes

About 90–95% of all instances of diabetes are T2DM, often referred to as non–insulin-dependent diabetes mellitus (NIDDM) or maturity-onset diabetes in the former nomenclature. Insulin resistance and β-cell dysfunction are the two main insulin-related disorders that define this form of diabetes. The ability of cells in peripheral organs to respond to insulin, particularly those in muscle, the liver, and adipose tissue. Reduced insulin sensitivity in the early stages of the illness causes β-cell hyperactivity, which in turn causes a compensatory increase in insulin production to maintain euglycemia (Meuleneire, 2008). As a result, hyperinsulinemia, or high amounts of circulating insulin, prevents hyperglycemia. Increased insulin release by β cells cannot fully compensate for decreased insulin sensitivity. Additionally, the -cell activity starts to deteriorate, and -cell malfunction finally results in an insulin shortage. Hyperglycemia results from the inability to maintain normal blood sugar levels (Banday et al., 2020). Although insulin levels drop, insulin production usually suffices to stop the onset of DKA. DKA can also be caused by the use of certain medications such as sodium-glucose cotransporter-2 (SGLT2) inhibitors, corticosteroids, and atypical antipsychotics (second-generation antipsychotics). Under conditions of physiological stress, patients with type 2 diabetes often do not in need insulin therapy at the onset of the disease or throughout life.

Because T2DM develops very slowly and asymptomatically, mild hyperglycemia progresses over years, leading to severe symptoms such as weight loss, growth retardation, blurred vision, polyuria, and polydipsia. It is usually not diagnosed until the typical symptoms of hyperglycemia appear. The etiology/pathogenesis of this form of diabetes is complex and includes a number of known and unknown factors that can be fully explained by a combination of genetic

predisposition (polygenic) and strong influence strongly from the environment (Banday et al., 2020).

T2DM is often associated with increasing age, obesity, family history of diabetes, physical activity, and acceptance of modern lifestyles. Previous GDM and pathophysiological conditions such as hypertension and dyslipidemia in women. It is more common among people of certain races or ethnic groups, such as Native Americans (American Indians), Asian Americans, African Americans, Hispanics, and Latinos. High incidence of T2DM in certain racial or ethnic people and the strong association seen with first-degree relatives suggest a role for genetical factors in the pathogenesis of this disease, but all these factors remain complicated and largely undefined (Meuleneire, 2008). However, unlike T1DM, the disease is not linked to genes involved in immune responses, including autoimmunity, leading to immune-mediated pancreatic β-cell destruction.

Obesity plays a critical role within whole-body glucose homeostasis, as it affects the development of insulin resistance through its effects on tissue sensitivity to insulin. Not all are overweight or obese. The increased body fat percentage characteristic of obesity is an important risk factor for type 2 diabetes, and not only total body fat mass but also distribution determine the development of insulin resistance and subsequent hyperglycemia (Banday et al., 2020). Increased abdominal fat or visceral obesity is more often associated with this type of diabetes than increased gluteal/subcutaneous fat or peripheral obesity. Patients with T2D frequently have different cardiovascular risk factors such hypertension and metabolic abnormalities due to the significant relationship between increased body fat content or obesity. High levels of triglycerides and low levels of high-density lipoprotein (HDL) are indicators of lipoprotein metabolism (Meuleneire, 2008). Due to its longevity and therefore the various associated

metabolic disturbances characteristic of hyperglycemia, T2D, especially within the middle and later decades, is commonly related to the event of microvascular complications and different circuits. It depicts a number of the foremost risk factors for type 2 diabetes.

1.2.2. Pathophysiology of Obesity

Controlling energy use and consumption is the primary mechanism for achieving energy balance. One calorie is actually one calorie and all calories are equal in this fundamental energy calculation. However, when we move past this purely energy-based perspective and consider the pathophysiology of obesity-related comorbidities, we see that not all calories are created equal. A thorough explanation of the pathophysiology of obesity should thus contain two simultaneous discussions: one from an energy point of view and the other from a nutritional point of view. Here, we primarily concentrate on the former since, in contrast to the composition of the energy balance, there is still misunderstanding and disagreement over the processes governing it is best nutrition (Mozaffarian & Ludwig, 2015) (De Souza et al., 2012).

The contrast between obesity's causes and consequences must be properly taken into account, as must the significance of comprehending the pathophysiology of comorbidities, such as cardiovascular disease, that is both independent of and reliant upon obesity.

Most scientists concur that body weight or fatness is actively controlled or protected based on the data that adult body weight is extraordinarily stable and unaffected by short-term up or down experimental disruptions under consistent environmental settings (Schwartz et al., 2017). The growing amount of evidence supporting the idea that obesity is a disease and that high body weight/fat in many obese patients is just as protective as in normal weight subjects shifts the blame for obesity from humans to physiology (Hall & Guo, 2017). With the discovery of more

than 140 chromosomal regions linked to obesity, data from genome-wide association studies indicate a hereditary susceptibility to fat (Fall et al., 2017). The central nervous system "8" often had a high gene expression of BMI and fatness (Locke et al., 2015). Only a small number of genes, nevertheless, have been found to have a significant impact on BMI with paternally expressed genes along a particular area of chromosome 15 that produce Prader-Willi syndrome, these genes also encode leptin and melanocortin signaling elements (Angulo et al., 2015). Contrary to these single-gene examples, it is believed that widespread obesity is caused by a large number of genes with a small impact size.

Most people agree that environment, lifestyle, and genetic predisposition combine to cause obesity. The existence of genes that increase the risk of being obese has been the subject of several theories. According to the "thrifty" gene hypothesis, throughout the course of human evolution, genes that encourage energy intake and high fuel efficiency were preferred above those that encourage energy expenditure. According to the "drifting" gene concept, after humans developed weapons and guns some 2 million years ago and were no longer in danger from predators, there were evolutionary selection pressures for genes that limited weight/obesity. It is said to be moderated and causes genes to randomly wander (Speakman, 2008).

According to theories concerning the early onset of the disease in adults, children of mothers who had metabolic issues including malnutrition, obesity, or diabetes may grow up with obesity (“Developmental Origins of Adult Health and Disease,” 2004). The epigenetic regulation of genes through methylation, histone modifications, chromatin remodeling, and noncoding RNA alterations is one of the molecular processes involved in early metabolic programming (Cordero et al., 2015). The increased epigenetic risk of adult obesity is significant because it may be passed on to next generations, increasing the obesity epidemic. Therefore, one of the main

objectives of obesity research is to develop methods and therapies to stop the negative effects of epigenetic programming.

We propose that obesity genes are not only within hypothalamic homeostasis regulators of energy balance, but also neural circuits involved in their interaction with obesity, given the disproportionately high expression of obesity-associated genes and their epigenetic modifications in the central nervous system. settings, including the circuits that are presumably involved in reward-based decision-making, learning and memory, delay discounting, and spatial orientation (Gadde et al., 2018).

1.3.1.1. Treatment options currently available

Table 2:Different drugs of diabetes and obesity with mechanism of action

Drug	Mechanism of action	Reference
1. LEVEMIR	The modulation of glucose metabolism is insulin detemir's main function. Insulins, such as insulin detemir, work specifically by attaching to insulin receptors. Receptor-bound insulin reduces blood sugar by promoting the absorption of glucose into cells.	(*Levemir® (Insulin Detemir [RDNA Origin] Injection)*, n.d.)

	By preventing the liver from releasing glucose, skeletal muscle, and fat are affected. Insulin blocks adipocyte lipolysis increases protein synthesis while reducing proteolysis.	
2. Tresiba	Insulin degludec, an active component in Tresiba, controls glucose metabolism similarly to other forms of insulin. By promoting peripheral glucose absorption and limiting hepatic glucose synthesis, it lowers blood glucose levels.	(*Tresiba (Insulin Degludec) for the Treatment of Type 1 and Type 2 Diabetes - Clinical Trials Arena*, n.d.)
3. Contrave	The drug Contrave combines the opioid antagonist naltrexone with the dopamine and norepinephrine reuptake inhibitor bupropion. Nonclinical research indicates that naltrexone and bupropion affect the hypothalamus (the brain's center for controlling appetite) and the mesolimbic dopamine circuit, two distinct regions involved in controlling food intake (reward system)	(*Contrave (Naltrexone HCl and Bupropion HCl) | CenterWatch*, n.d.)

1.3.1.2. Next-Generation Insulin Analogs

Insulin is a prodigy substance, but a threatening drug. This is the first-choice treatment for type 1 diabetes and type 2 diabetes in its advanced stages, this is the recommended course of therapy. We have steadily improved insulin quality and manufacturing over the past ten years, allowing for nearly infinite biosynthesis of the purest possible chromatographic insulin. Site-specific alterations that speed up or slow down insulin activity have been introduced via biosynthesis to enhance hormone pharmacokinetics (Hirsch, 2005). As a result, pharmacodynamics has replaced pharmacokinetics as the primary objective of cutting-edge research. Like incretins, which are only active in hyperglycemia, finding insulin that is sensitive to glucose is an important objective.

Such insulin analogs or new formulations offer a more aggressive treatment of hyperglycemia while reducing the risk of life-threatening hypoglycemia (Chabenne et al., 2014; Wu et al., 2011). At the same time, novel glucagon formulations and structural analogs are being created, along with continuous glucose monitoring, in an effort to preserve the integrity of injected insulin (Sociali et al., n.d.). It is not difficult to envisage that in the not-too-distant future, significantly better strategies for insulin-dependent glycemic control may be developed.

Despite this, efforts to combine insulin treatment with weight loss have progressed to a significant degree. accelerates pancreatic dysfunction while fostering basal insulin treatment with a GLP-1 agonist, which has been clinically shown to reduce hypoglycemia and weight gain and enhance glycemic control. This is a paradigm change since it suggests that attempts to further reduce the need for insulin will be boosted by discovering new pathways that improve insulin sensitivity and endogenous beta-cell activity(Elia et al., 2015).

1.3.1.3. Pancreatic Transplantation

Despite the fact that pancreas transplantation is not a novel surgery, significant improvements in its development and success rate have made these invasive therapies more alluring. For T1D patients having kidney transplants, those with poorly managed blood sugar levels, or those who have recurrent hypoglycemia, surgery is a curative option that is frequently employed. One-year survival rates of over 95% and transplant survival rates of about 85% are evidence of advancements in immunosuppressive medication and transplant surgery, respectively(A. C. Gruessner & Sutherland, 2005).

Importantly, a successful transplant improved glycemic control over insulin treatment while lowering his HbA1c levels. Islet transplantation is a less invasive option to pancreas transplantation. Despite the obvious promise of a less invasive technique, pancreas transplantation often results in better long-term glycemic outcomes than islet transplantation (R. W. G. Gruessner & Gruessner, 2013). Obtaining sufficient numbers of human islets remains an ongoing challenge, and stem cell technology has great potentially fill the need. While resolving many safety issues connected to the possibility of uncontrolled growth and insulin release that might eventually evolve into a non-glucose-controlled evolution, there are still significant obstacles in scaling the technology for commercial usage increase (Elia et al., 2015).

1.3.1.4. Multi-hormone Combination Therapies

It is becoming more and more obvious that individualized enteroendocrine responses play a role in the significant and quick metabolic benefits brought about by bariatric surgery. Furthermore, current preclinical and clinical developments show that simultaneous targeting of various biological systems might improve metabolic effectiveness and decrease side effects in

comparison to traditional monotherapies(Sadry & Drucker, 2013). By delivering two distinct hormones at the same time, various metabolic pathways may be targeted concurrently (Cegla et al., 2014) or using unimolecular poly agonists. Together, these multifunctional hormones decorate specific hormonal action profiles but, more importantly, help adopt different pharmacology leading to improved efficacy and safety. In animal models of obesity and glucose intolerance, this peptide, a co-agonist that operates on glucagon and her GLP-1 receptors, was discovered in 2009 and shown to significantly reduce body weight and improve glucose metabolism (Day et al., 2009; Pocai et al., 2009). GLP-1/glucagon co-agonism was observed to reverse leptin resistance in DIO rats in a subsequent investigation. What proportion of weight reduction restores leptin activity in people is the key question at the center of this interesting finding, which paves the way for further clinical studies. Glucagon and the GLP-1 receptor in particular. A new human research exploring the effectiveness of simultaneous agonizing acts revealed encouraging metabolic gains. Although their ability to cause weight gain has impeded the development of GIP agonists for diabetes, innovative dual incretin core agonists (GLP-1/GIP) are efficacious in rodents and non-human primates. Improves insulin secretion and glycemic management, according to recent reports.

Additionally, clinical trials discovered that the coagonist's improved insulinotropic impact caused HbA1c levels to significantly improve at the maximum dose in just 6 weeks (1.1% from baseline). It's significant to note that co-agonist therapy was not connected to vomiting or changed intestinal motility. This implies that co-agonists can be used to boost effectiveness while retaining a high level of safety. Clinical investigations that will assess the effectiveness and security of these single-molecule co-agonists are now being conducted.

Numerous novel therapy procedures may be developed by extending the idea of utilizing multi-agonists or co-administering various drugs with complimentary modes of action. Therefore, this approach can greatly improve individualized treatment's potential to bridge the performance gap between pharmacotherapy and surgical intervention (Elia et al., 2015)**.**

1.3.2. Obesity

The best way to treat obesity is to have a healthy food routine, control-calorie diet and habit of exercise regularly. To do this one should:

1.3.2.1. Dieting

One of the most common meal plans is one that restricts calories. A very low-calorie diet has less than 800 calories, whereas a low-calorie diet has a total calorie consumption of between 800 and 1500. When you diet intensively, your basal metabolic rate decreases and your resting energy expenditure decreases. As a result, he needs less calories per day to maintain his weight after dieting. A normal diet with a slowed metabolic rate tends to promote post-diet weight gain. Physiologically, dieting is harmful, and after rapid weight loss, it takes years for the body to readjust back to its original weight. In recent research of 14 Biggest Loser contestants, it was shown that over the course of 30 weeks, participants shed an average of 128 pounds and were seen to have a lower resting metabolic rate (Fothergill et al., 2016). Those who lost the most weight had the lowest metabolic rate. Nearly all or most of the weight the participant lost was recovered in 5 of 14 participants in her 6 years after the program, and her metabolic rate was low despite the weight gain. As of yet, metabolic changes brought on by fast weight reduction

continue over time, indicating a flawed but proportionate reaction to ongoing weight loss efforts. Food is used as a pleasure or a punishment for dieting, and a problem with food fixation can arise. Hunger that is not met might cause mood changes and overeating. When eating is restricted despite drinking enough water, additional issues including dehydration and constipation might arise (St-Onge et al., 2017). It frequently makes unhealthy behaviors worse.

Additionally, dietary therapies have been suggested as anti-obesity measures. Using knowledge on dietary interventions such macronutrients, micronutrients, and nutritional biochemistry as a foundation for intervention design, Laura E. Matarese wrote a very instructive study in 2016 (Longo et al., 2019). This study discussed the use of metabolic theories, such as low-carb, ketogenic, or low-fat diets, to manage obesity. The majority of these suggested diets lack sound scientific foundations. Foods contain a wide range of macronutrients, and since dietary needs are not well defined, it is challenging to directly evaluate various weight reduction programs (St-Onge et al., 2017).

Specific nutrients shown to function pharmacologically to induce weight reduction were also investigated in addition to addressing nutritional deficits. For instance, physiologically active chromium (III) promotes fat and carbohydrate metabolism. There is proof that chromium can improve lean body mass, lessen cravings for carbohydrates, and assist control appetite. have been suggested, and other theories have been put out to look into this suggestion. The evidence for the reduction of body weight or fat mass by dairy products or calcium supplements is currently weak. Intermittent fasting is a different approach to calorie reduction that has also been proposed(Sangiao-Alvarellos et al., 2022).

1.3.2.2. Exercise

In addition to dietary changes, regular exercise also provides other benefits. You may enhance the advantages of routine exercise in maintaining weight reduction by boosting the satiety impact of solid meals and meals. Numerous advantages of exercise exist for controlling obesity (Bouchard et al., 1993) Whether you have never exercised before or not, this is true. New exercisers might not know how to perform some activities properly, which might result in harm. Prior to beginning an exercise program, it is important to have a thorough consultation with a physician and work with a skilled trainer or coach to create a program that is suited to each person's needs. Furthermore, exercise reduces appetite. But a lot of individuals use exercise as an excuse to eat bad things. People need to be mindful of the "halo effect." When someone starts an exercise regimen, poor diet may sabotage all physical effort. 12 miles a week of vigorous-intensity running, moderate-intensity jogging, or walking. (20 weekly miles). Volunteers were instructed to continue eating normally during the experiment.

According to the study's findings, those who participated in high-intensity exercise regimens lost abdominal fat, whereas people who participated in low- and moderate-intensity exercise regimens did not (Slentz et al., 2005). and the type of exercise program suggested by the trainer or physician. In reaction to changes in the amount of energy available, mechanisms for storing energy and controlling body weight are carefully regulated to ensure viability. This may be seen in the metabolic adaptability of the system to both famine and overeating. When these processes become rigid as a result of caloric restriction or increased energy intake, certain metabolic diseases can develop (Vettor et al., 2020).

After introducing a low-carbohydrate ketogenic diet to volunteers who were overweight and obese, researchers measured their energy expenditure. The study's findings demonstrated a

considerable increase in energy expenditure while going from a high-carbohydrate diet to a ketogenic diet, as determined by doubly-labeled water. This, however, was only perceptible on the days when patients were not housed in the metabolic room. Increased physical activity as assessed by an accelerometer could not explain this result(Sangiao-Alvarellos et al., 2022).

1.3.2.3. Probiotic /Prebiotic Supplementation

The health and allied sectors concentrate on the gut microbiota, probiotic bacteria (probiotics), and prebiotics in addition to exercise and nutrition to give therapies for obesity issues. Probiotics are living bacteria that provide the host with health benefits when taken in sufficient quantities. Improves barrier function and immunomodulation when particular bacteria from eating food with active antibacterial activity are used. The use of these microorganisms can greatly affect the management of obesity without the adverse effects associated with typical pharmacological treatment, which helps to minimize the inflammatory response. There have also been reports of specific stress effects on body weight and metabolism connected to probiotics. However, more investigation is necessary to pinpoint the strains that could have advantageous benefits.

Therefore, it is not yet possible to endorse their systematic usage in the management of obesity. Prebiotics often serve as a food supply for probiotics, or good bacteria, in the digestive system. Prebiotics are essentially carbohydrates that are indigestible to humans. They function as biological agents, controlling the production of cytokines and improving the absorption of certain ions and trace elements. The use of probiotics and prebiotics is only one component of a weight reduction plan; additional research is needed to determine the dosage, duration, and long-term effects(Cerdó et al., 2019). Probiotic and prebiotic therapies should start early in life to

avoid obesity and its effects as people mature. Additionally, due to the increased risk of infection when using pre/probiotics, immunocompromised patients should proceed with caution when eating them(Sangiao-Alvarellos et al., 2022).

1.3.2.4. Drugs

Along with a healthy diet, consistent exercise, and the use of pre- and probiotics, certain weight reduction medicines are accessible with a prescription after consulting a doctor, while less effective treatments are also on the market. Others can be found in shops and on commercial websites. Orlistat (Xenical, Alli), phentermine-topiramate (Qsymia), naltrexone-bupropion (Contrave), liraglutide (Saxenda), and semaglutide (Wegovy) are five medications that have been authorized by the FDA for long-term usage. The sixth treatment for those who have been identified as having one of three uncommon genetic illnesses is setmelanotide (IMCIVREE). Genetic testing is needed to confirm the diagnosis. As long as the medication is effective and has no negative side effects, these medications may be utilized. According to reports, anti-obesity medications might seriously harm the liver. Daily multivitamins are frequently given in order to make sure that those taking the tablets receive adequate vitamins that their diets cannot provide.

Patients who have high blood pressure, epilepsy, anorexia nervosa, renal illness, mental disorders, are pregnant or want to become pregnant, are nursing, or have any of these conditions should consult a doctor. see a doctor before using any anti-obesity medication. FDA-approved appetite suppressing medications are only permitted for short-term usage, often up to 12 weeks. Very few studies have looked at whether they are secure and efficient over the long run, despite some healthcare practitioners prescribing them for extended periods of time. (*Prescription Medications to Treat Overweight & Obesity | NIDDK*, n.d.). Anti-obesity medications have shown short-term

efficacy in epidemiological studies, but long-term contraindications. Recalled medications appear to be increasing exponentially in the pharmaceutical sector.

The history of prescription medications reveals that they have resulted in millions of fatalities and billions of dollars' worth of damage. As a solution, the pharmaceutical industry now only appears to be able to offer medications that balance the equations relating to weight management and energy metabolism. In an effort to address the obesity pandemic, researchers are creating new medications and changing the doses of current medications that have not been successful. For some groups, even the most recent "Semaglutide" diet medication that the FDA authorized in 2021 has restrictions. Notably, clinically meaningful weight reduction in overweight or obese people receiving 2.4 mg once weekly of semaglutide was related with both a lifestyle intervention (rather than just medicine). (Sangiao-Alvarellos et al., 2022) (*Prescription Medications to Treat Overweight & Obesity | NIDDK*, n.d.).

1.4. Rationale of the study

Sirt6 is a member of the sirtuin family which is a kind of NAD+-dependent histone deacetylase and ADP-ribose transferase enzyme. It controls aging, cancer, obesity, insulin resistance, inflammation, and energy metabolism, playing a significant role in both physiological and pathological processes. Reduced Sirt6 activation may be linked to diabetes and obesity, according to recent research. Sirt6 levels and function diminish with age and overnutrition, two key risk factors for obesity and diabetes, which results in improper glucose and lipid metabolism. Mice with Sirt6 ablation across the body have acute hypoglycemia. Sirt6 deficiency enhances diet-induced obesity and insulin resistance and causes hepatic steatosis Diabetes and obesity are

protected against Sirt6.This review examines the data supporting Sirt6's emerging function as a regulator of metabolism in mammals and lists its key roles in obesity and diabetes.

1.5. Aim and objectives of the study

The aim of the current study is to investigate the association of SIRT6 with diabetes as well as in obesity. To evaluate the expression levels of sirtuin 6 in subcutaneous adipose tissue collected from overweight and obesity. Sirtuins (SIRT) are a family of conserved enzymes which are able to affect many metabolic and inflammatory pathways thereby potentially improving health and increasing lifespan.

Chapter 2

Methodology

The current study was focused to determine an implication between SIRT6 in diabetes and obesity to identify the most promising target for complete and fruitful eradication of diabetes and obesity. The information from this review paper was collected from various primary sources such as Google Scholar, Research Gate, Nature, NCBI, Science Direct, Elsevier and Springer,etc. Information is also collected from secondary research articles such as PubMed, Frontiers, and Medline, etc. After scrutinizing necessary information from all the articles an outline was created to present the information sequentially. First, it was important to discuss about Pathophysiology of diabetes and obesity. Later, a further literature search was performed to gather information on the significant implication between SIRT6 and diabetes and obesity. Finally, targeting sirtuins for the treatment of diabetes, an effective therapeutic target was established for the fruitful eradication of the specific diseases. For conducting the whole write-up, a piece of valid information was collected, and an accurate citation was done with careful consideration

Chapter 3

Association of SIRT6 with diabetics and obesity

3.1 Role of SIRT6 in diabetics

Since numerous studies have demonstrated SIRT6's crucial roles in metabolism, it has recently attracted a lot of attention. In important tissues that produce insulin and those that it targets like adipocytes, pancreatic cells, skeletal muscle, and kidneys, Insulin resistance and local and systemic energy metabolism are both regulated by SIRT6. In view of animal studies undertaken to create new and effective diabetes medications, SIRT6 can be seen as appealing in the metabolism of glucose as a target. In a prior investigation, it was discovered that animals lacking SIRT6 had increased tissue glucose absorption, high expression of the glucose transporter GLUT1, and induced hypoglycemia (Klionsky et al., 2016). Without SIRT6, increased glycolysis and suppressed mitochondrial respiration result in excessive glucose (Sociali et al., n.d.) consumption (Kugel & Mostoslavsky, 2014). HIF1-, a crucial glycolytic regulator, regulates a number of genes, including lactic acid dehydrogenase (LDH), triose-phosphate isomerase (TPI), GLUT1, pyruvate dehydrogenase kinase 1 (PDK1), pyruvate dehydrogenase kinase 4 (PDK4), and phosphofructokinase 1 (PFK1). It is important to highlight that SIRT6 has been shown to interact with and repress the critical glycolytic regulator HIF1. As a result, the deletion of SIRT6 increases the transcription of glucose transporters and glycolytic enzymes through HIF1. Anti-diabetic medications may therefore aim to inhibit SIRT6, which could therefore result in glycolysis and glucose reuptake. Additionally, SIRT6 has been seen to control the process of

gluconeogenesis; Reduced glucose synthesis is linked to lower SIRT6 levels. Reduced levels of SIRT6 lead to an increase in the acetylation of the PGC-1 alpha protein, which in turn causes the general control non-repressed protein 5 to be inactivated and lowers the expression of the gluconeogenic genes(Dominy et al., 2012). SIRT6 may be involved in glucose regulation since SIRT6 depletion results in hypoglycemia by increasing insulin signaling and activating protein kinase, protein kinase B (Akt). SIRT6 controls the expression of two genes, pancreatic and duodenal homeobox 1 (Pdx1) and glucose transporter 2 (Glut2), to regulate pancreatic cells' capacity to sense glucose and their ability to tolerate systemic glucose(Friedman & Appel, 2019). Forkhead box protein O1 is deacetylated by SIRT6 in pancreatic cells. (FoxO1)protein (Akter et al., 2021).

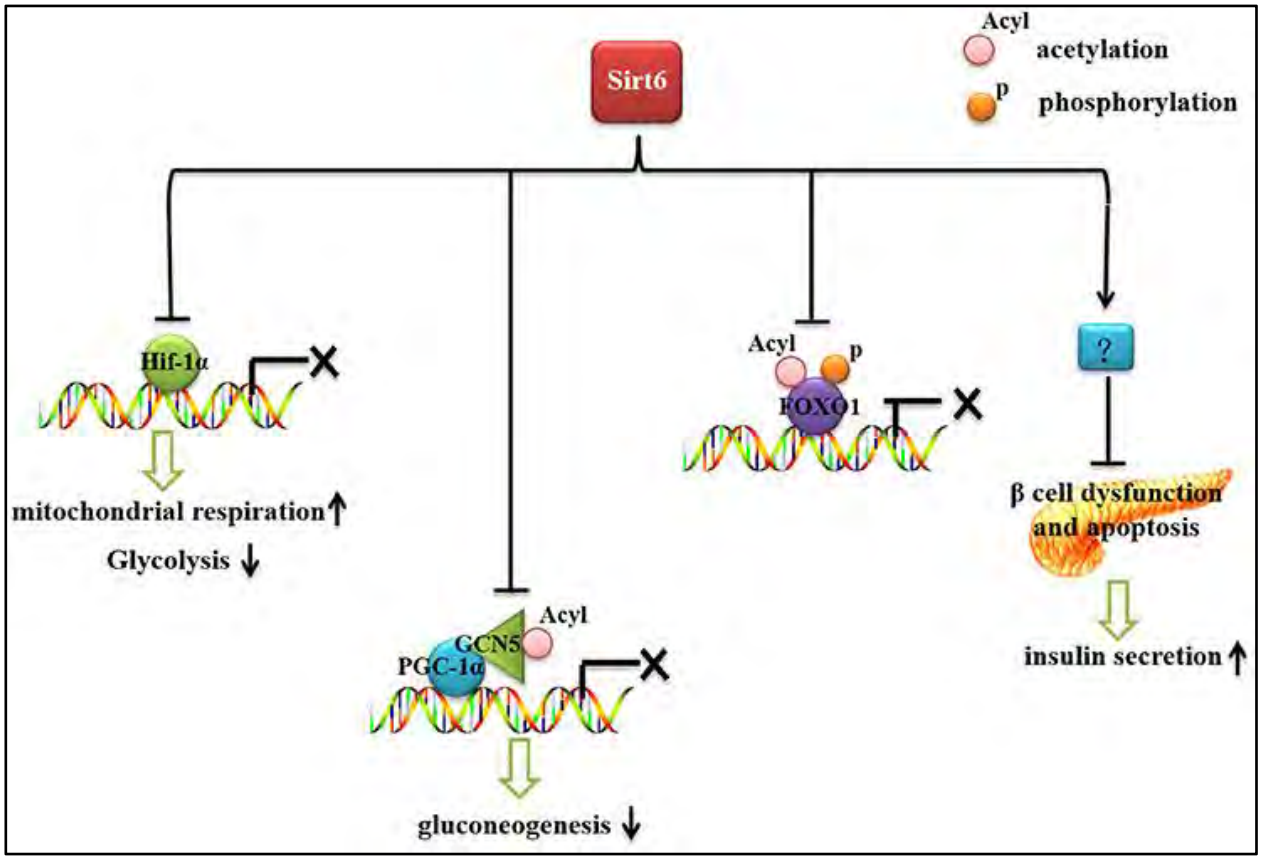

Figure 2: Role of Sirt6 in glucose metabolism

FIGURE 2: The role of Sirt6 in glucose metabolism. Sirt6 inhibits recruitment of Hif-1α to its target gene promoters, increases mitochondrial respiration and inhibits glycolysis. Sirt6 binds to and activates GCN5, inhibits the acetylation of PGC-1α, and decreases the expression of gluconeogenic genes. Sirt6 can specifically interact with FoxO1, decrease FoxO1 acetylation and phosphorylation level, and inhibit the interaction between FoxO1 and its downstream gene promoters, thereby reducing the expression of gluconeogenic genes. Sirt6 protects against β-cell dysfunction and apoptosis and increases insulin secretion.

3.1. 1. SIRT6 and Blood Glucose

The severe hypoglycemia phenotype seen in Sirt6-deficient mice provided the first indication that Sirt6 may be involved in glucose metabolism(Mostoslavsky et al., 2006). Mice lacking Sirt6 displayed enhanced insulin signaling and glucose absorption across their entire body (Xiao et al., 2010). The Sirt6-deficient mouse is young, but seems normal for the first two weeks. After that, the mouse starts to show a variety of acute degenerative phenotypes, and at around one month old, it passes away.

Lymphopenia, osteopenia, lordosis, and reduced subcutaneous fat are phenotypes (Mostoslavsky et al., 2006). Severe hypoglycemia is the most noticeable trait and may have a significant role in the mortality of mice before their first month of life. However, because blood insulin levels are much lower than in wild-type mice, it is possible that the severe hypoglycemia phenotype is not brought on by aberrant insulin levels (Xiao et al., 2010). It is found that mice given 10% glucose

in water had higher blood glucose levels and that 83% of mice with systemic Sirt6 deficiency survived, proving that hypoglycemia is a significant contributor to postnatal mortality. It was finished. Xiao et al. discovered that Sirt6 inhibits a number of molecules upstream, insulin receptors, and insulin receptor substrates 1 and 2 to adversely regulate Akt phosphorylated at Thr 308 and Ser 473. (Xiao et al., 2010).

Insulin signaling is stimulated and Akt phosphorylation is enhanced with Sirt6 deficiency (Xiao et al., 2010). Increased glucose absorption has the potential to result in hypoglycemia. However, a lack of Sirt6 did not impact the gut's ability to absorb glucose, nor did it cause greater blood sugar levels or renal excretion. (Zhong et al., 2010). Increased glucose absorption was observed in brown adipose tissue and muscle, but not in the liver, brain, or heart, in an in vivo 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) investigation of Sirt6-deficient animals. This finding may help to explain the hypoglycemic phenotype. (Zhong et al., 2010). The enhanced expression of glucose transporter 1 (Glut1), one of the principal glucose transporters that controls basal, independent glucose absorption, may account for the increased glucose uptake in these organs. depending on growth factors or insulin (Zhong et al., 2010).

The control of glucose metabolism is also significantly influenced by the central nervous system. Similar to what was shown in upper brain-specific Sirt6-inhibited animals, levels of growth hormone and insulin-like growth factor-1 (IGF-1) were lower in brain-specific Sirt6-inhibited mice than in control mice (Schwer et al., 2010). Thus, Sirt6 may influence insulin sensitivity and glucose metabolism through growth hormone/IGF-1 transmission under specific physiological circumstances.

3.1.2 SIRT6 and Insulin-Sensitive Organs

SIRT6 expression rose with weight reduction in human adipose tissue but reduced in adipose tissue from db/db mice, indicating a possible function for SIRT6 in adipose tissue (Dominy et al., 2012) (Moschen et al., 2013). By reducing the expression of kinesin family member 5C (KIF5C) and subsequently raising kinase CK2 activity, Chen et al (Chen et al., 2017). showed that SIRT6 is necessary for mitotic lineage expansion during adipogenesis. Insulin resistance and obesity brought on by the HFD are not as easily produced in Sirt6 transgenic miceSirt6-specific fat removal, on the other hand, made mice more susceptible to obesity and insulin resistance brought on by the HFD and raised blood glucose levels and fatty liver disease. The subset of PPAR target genes involved in lipid metabolism, lipid transport, and adipogenesis are upregulated when SIRT6 is overexpressed(Kanfi et al., n.d.).

In instance, SIRT6 enhanced blood triglyceride clearance and lowered triglyceride production in adipose tissue while decreasing the expression of diglyceride acyltransferase 1 (DGAT1), a key enzyme in triglyceride synthesis, and ANGPTL4, a negative regulator of lipoprotein lipase. By raising FoxO1's acetylation and phosphorylation and decreasing the production of adipose triglyceride lipase (ATGL), a crucial lipolytic enzyme (Kanfi et al., n.d.), Sirt6 removal decreases FoxO1's transcriptional activity, which in turn decreases the adipose lipase process lipolysis. Brown adipocytes' thermogenic function is impaired by fat-specific inactivation of Sirt6, which also causes obesity and insulin resistance. It was discovered that Sirt6 deletion decreased ATF2's ability to bind to the PGC-1 promoter, which in turn decreased the expression of the target thermogenic genes PGC-1 and PGC-1 (Yang et al., 2018).

3.1.3 SIRT6 Represses Gluconeogenesis and Lipid Accumulation in the Liver

Sirt6 affects gluconeogenesis as well as controlling glycolysis. Hepatic gluconeogenesis is considerably enhanced in the absence of Sirt6, indicating a compensatory response to hypoglycemia(Dominy et al., 2012). Numerous cell signaling pathways and transcription factors carefully control the gluconeogenic process (Locke et al., 2015). The transcriptional regulator of gluconeogenesis, peroxisome proliferator-activated receptor gamma-coactivator 1 (PGC-1alpha), is important. PGC-1alpha stimulates the expression of gluconeogenic enzymes such phosphoenolpyruvate carboxykinase (Pepck) and glucose-6-phosphatase (G6p).

PGC-1alpha's degree of acetylation is a negative regulator of its transcriptional activity. PGC-1 alpha transcriptional activity was reduced and PGC-1 alpha acetylation levels were elevated when general regulatory protein 5 (GCN5) was not suppressed. GCN5 can be directly bound to by Sirt6 and activated (Dominy et al., 2012). Sirt6 deletion decreases GCN5 activity, decreases PGC-1alpha acetylation levels, and PGC-1 alpha controls the expression of the gluconeogenic genes (Dominy et al., 2012).

The forkhead box protein O1 (FoxO1) is involved in the control of gluconeogenesis as well. FoxO1 directly binds to the G6p and Pepck promoter areas to initiate gluconeogenesis (Weyrich et al., 2008). The FoxO1 transcriptional activation domain mutation and abrogated activity led to a significant reduction in gluconeogenesis. Lack of FoxO1 significantly reduced the expression of G6p and Pepck brought on by fasting. Phosphorylation and acetylation of FoxO1 play a major role in controlling its transcriptional activity (Brunet et al., 2004; Yamagata et al., 2008; Zhao et al., 2010).

In Sirt6-deficient cardiomyocytes, FoxO1 phosphorylation was elevated. FoxO1 is phosphorylated, which inhibits its transcriptional activity and encourages FoxO1 translocation from the nucleus to the cytoplasm. Following research, it was shown that Sirt6 specifically interacts with FoxO1, limiting the interaction between FoxO1 and its downstream genes G6p and Pepck, resulting in a reduction in the production of gluconeogenic genes (Klionsky et al., 2016).

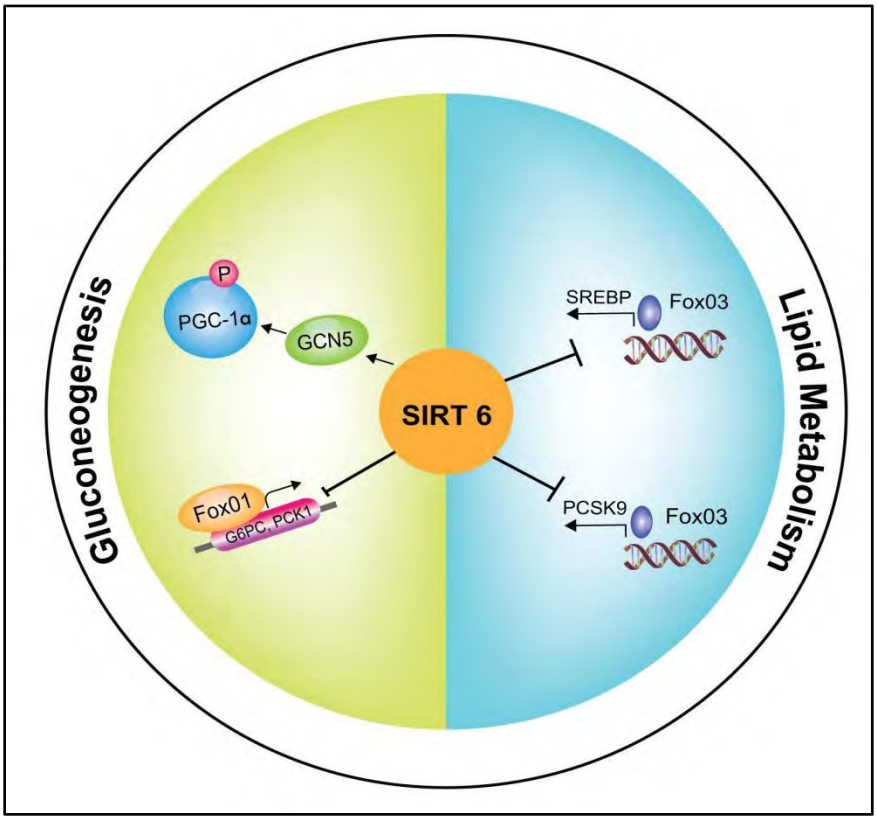

Figure 3:Gluconeogenesis and Lipid metabolism.

Additionally, SIRT6 controls lipid metabolism via a number of ways. Reduced -oxidation, increased triglyceride buildup, and the development of fatty liver were all outcomes of liver-specific deletion of SIRT6 in mice, albeit the molecular mechanisms underlying these effects have not yet been fully uncovered.

This suggests that SIRT6 may have a function in the setting of the human illness as human fatty liver samples had lower levels of SIRT6 than normal controls. When exposed to a high-fat diet (HFD), animals overexpressing SIRT6 have decreased levels of visceral fat, low-density lipoprotein (LDL) cholesterol, and triglycerides. As was already established, SIRT6 inhibits KIF5C to induce the mitotic clonal growth required for adipogenesis.

Additionally, SIRT6 inhibits cleavage of SREBP1 and SREBP2, which remained inactive, and increases phosphorylation and inhibition of SREBP1 in an AMPK-dependent manner, which are three ways SIRT6 negatively regulates the lipogenic transcription factors SREBP1 and SREBP2. SREBP1 and SREBP2 regulate the expression of many genes, including 3-hydroxy-3-methylglutaryl-CoA reductase, to enhance cholesterol production.

A research demonstrates that FoxO3 binds SIRT6 to the promoter region of the SREBP2 gene, where SIRT6 deacetylates histone H3K9 and H3K56 and downregulates SREBP2. This supports its function in lipogenesis.The overexpression of either SIRT6 or FoxO3 improves hypercholesterolemia in a model of obese mice. In addition, SIRT6 was shown to modulate the circadian lipid profiles, working with BMAL1 and CLOCK, through the regulation of SREBP1 . In addition to down regulating SREBP1 and SREBP2, SIRT6 can downregulate PPAR-γ responsive genes and genes associated with lipid storage, such as angiopoietin-like protein 4, adipocyte fatty acid-binding protein, and diacylglycerol acyltransferase 1. As a result of these mechanistic functions of SIRT6, mice overexpressing Sirt6 exhibit lower LDL cholesterol levels and become protected against phenotypes related to obesity.

3.1.4. SIRT6 Increases Insulin Sensitivity in the Skeletal Muscle

SIRT6 is involved in regulation of metabolic balance in skeletal muscle. Enhanced insulin sensitivity in skeletal muscle and increased insulin-induced Akt activation in the gastrocnemius are both seen in Sirt6 transgenic mice skeleton-specific muscles (Chen et al., 2017). On the other hand, sirt6 ko mice have impaired glucose homeostasis and insulin sensitivity, which leads to a reduction in whole-body energy expenditure (Chen et al., 2017). Sirt6 deletion decreases AMPK activity, which in turn decreases the expression of genes involved in fatty acid oxidation, mitochondrial oxidative phosphorylation, and the absorption of glucose and lipids. The direct mechanism behind SIRT6 action in skeletal muscle requires more study (Yang et al., 2018).

3.2 Role of SIRT6 in obesity

Sirt6 deficiency enhances diet-induced obesity and insulin resistance and causes liver steatosis. Diabetes and obesity are protected against Sirt6. This review examines the data supporting Sirt6's emerging function as a regulator of metabolism in mammals and lists its key roles in obesity and diabetes.

3.2.1 Metabolic effects of sirtuins

SIRT1 is the most studied sirtuin with metabolic effect. Because of its similarity to the yeast Sirt2 protein, which has been demonstrated to lengthen yeast life when overexpressed, SIRT1 initially generated a lot of enthusiasm. SIRT2 homologs in Drosophila melanogaster demonstrated similar life-extension effects at first. There was a lot of optimism that SIRT1,

which is the closest mammalian homolog to yeast SIRT2, may similarly contribute to human longevity. The benefits of SIRT1 on extending life in C. elegans and D. melanogaster, however, have lately come under scrutiny (Huynh et al., 2013).

Furthermore, it is uncertain whether SIRT1 is necessary or sufficient to lengthen the lifetime of animals because some research links SIRT1 to a longer lifespan while others find no association. The influence SIRT1 has on preventing age-related diseases, such as Type 2 diabetes, is more obvious than its questionable role in extending lifespan. The liver, muscle, adipose tissue, heart, pancreas, and brain are among the tissues that express SIRT1. Interestingly, two of the most common risk factors for Type 2 diabetes—diet-induced obesity and aging —both cause SIRT1 levels to drop in mice. Additionally, visceral adipose tissue SIRT1 expression is reduced in humans with morbid obesity.

These results suggest that decreasing SIRT1 expression may be one mechanism linking aging and obesity to the development of Type 2 diabetes (Huynh et al., 2013). Animals with a modest overall reduction in SIRT1 do grow obese, experience insulin resistance, accumulate more lipid in the liver, and exhibit increased inflammation in adipose tissue when fed a high-fat diet. According to these results, transgenic mice with SIRT1 whole-body overexpression are leaner, more metabolically active, and have lower caloric intake than control animals. Additionally, they showed improved glucose tolerance and reduced fasting blood levels of insulin, glucose, and cholesterol. When given a high-fat diet, mice with moderately elevated SIRT1 nevertheless exhibit improved fasting blood insulin and glucose levels as well as enhanced glucose tolerance. The molecular mechanisms behind SIRT1's anti-diabetic activities have been thoroughly investigated and recently reviewed (Huynh et al., 2013). These results collectively imply that

SIRT1 may be a therapeutic target for the management of diabetic symptoms as well as the potential defense against diet- and age-related diabetes.

While a substantial amount of research demonstrates the significance of SIRT1 in metabolism, SIRT3 and SIRT6 have lately drawn attention to their separate functions in preserving metabolic homeostasis. In addition to the liver, brain, kidney, skeletal muscle, and brown adipose tissue. These organs involved in metabolism also express SIRT3 (Kanfi et al., n.d.). One research that identified SIRT3 as a critical mitochondrial deacetylase showed a number of essential mitochondrial enzymes to be hyperacetylated in the liver, brown adipose tissue, heart, and brain of SIRT3-deficient mice. Under normal conditions, these mice displayed no metabolic abnormalities; nevertheless, when exposed to metabolic pressures including prolonged fasting and high-fat diet feeding, SIRT3-deficient animals developed several metabolic problems. Sirt3 is often increased in the liver and brown adipose tissue while people are fasting.

However, SIRT3-deficient mice did not have elevated SIRT3 when they fasted for 24hr, and this led to lower levels of fatty acid oxidation and higher levels of triglycerides in the liver (Yang et al., 2018). Contrary to fasting, it has been demonstrated that a long-term high-fat diet decreases the levels of SIRT3 mRNA, protein, and activity in the mouse liver, resulting in hyperacetylation and a reduction in the activity of mitochondrial proteins involved in oxidative metabolism (Xiao et al., 2010). Interestingly, acute high-fat eating elevated SIRT3 expression more than chronic high-fat feeding did. This molecular cause is unknown.

Physiologically, mice with a complete loss of SIRT3 displayed increased obesity, glucose intolerance, insulin resistance, hyperlipidemia, and steatohepatitis when fed a high-fat diet over an extended period. Several recent publications go into more detail about the molecular mechanisms behind these physiological changes. These results suggest that SIRT3 is crucial for

maintaining metabolic homeostasis, raising the possibility that individuals with Type 2 diabetes or symptoms of the metabolic syndrome may have lower levels of SIRT3. So, a therapeutic approach that stimulates SIRT3 could be a successful way to treat Type 2 diabetes (Chen et al., 2017).

SIRT6 also connected to metabolism, specifical to maintaining healthy glucose homeostasis, in addition to SIRT1 also to SIRT3. The nucleus is home to SIRT6, which has been linked to telomere maintenance, DNA repair, and aging. According to one study, SIRT6 knockout mice are more small than typical mice and exhibit significant metabolic problems, such as a lack of subcutaneous fat, severe hypoglycemia, and early death. An essential regulator of reactions to dietary stress, transcription factor Hif1 was found to have SIRT6 as a corepressor. An increase in Hif1 activity may be responsible for the hypoglycemia seen in SIRT6 knockout mice, which leads to increased glucose absorption and decreased mitochondrial respiration (Mostoslavsky et al., 2006). Furthermore, it was demonstrated that a lack of SIRT6 caused low levels of circulating insulin but increased insulin signaling, which also causes hypoglycemia. Interestingly, transgenic mice overexpressing SIRT6 displayed improved glucose tolerance when take a high-fat diet or aged to 19 months compared to wild-type controls, and this was accompanied by increased glucose-stimulated insulin secretion. Interestingly transgenic mice overexpressing SIRT6 had improved glucose tolerance when fed a high-fat diet or aged 18 to19 months compared to wild-type controls. This was accompanied by increased glucose-stimulated insulin secretion. These suggest that SIRT6 is essential in preserving healthy glucose homeostasis, and further research is needed to ascertain SIRT6's entire metabolic function and explore its potential as an anti-diabetic drug (Kanfi et al., n.d.).

The metabolic functions of the other sirtuins are less well understood than those of SIRT1, SIRT3, and SIRT6, respectively. It has been demonstrated that SIRT2 deacetylates and activates FOXO1, a transcription factor that controls both the genes for glucose and lipid metabolism, as well as deacetylates and inhibits the degradation of PEPCK, which is the rate-limiting enzyme in gluconeogenesis (Mostoslavsky et al., 2006). SIRT4 has been shown to have mild impacts on metabolism in two trials. Mice lacking SIRT4 exhibit higher plasma insulin levels induced by amino acids, somewhat lower fasting blood sugar, and slightly better glucose tolerance. Additionally, increased expression of genes involved in fatty acid oxidation in the liver and muscle, as well as a small drop in levels of glucose in blood, were the results of SIRT4 suppression in mice using tail vein injections of an siRNA adenovirus. The urea cycle is improved by SIRT5's ability to deacetylate and succinylated (Kanfi et al., n.d.) CPS1. SIRT7 has not yet been shown to have any effects on metabolism. More research must be done to comprehend the biochemical pathways regulated by SIRT2, SIRT4, SIRT5, and SIRT7. It is tempting to hypothesize that these other sirtuins could be targets for anti-diabetic drugs (Mostoslavsky et al., 2006).

3.2.2 Genetic polymorphisms in sirtuin genes

Numerous single nucleotide polymorphisms (SNPs) in the SIRT1 and SIRT3 genes have been linked to obesity and Type 2 diabetes in humans, indicating that sirtuins may be involved in the onset of these diseases. n a study of obese and lean people of Belgian Caucasian heritage, the SNP rs7069102 located in intron 4 of SIRT1 was connected to a substantial increase in visceral fat area among obese guys having one or two copies of the minor allele compared to obese men with the wild-type allele. (Peeters et al., 2008). In a study of 1279 Japanese individuals, the

identical SIRT1 SNP, along with SNPs rs7895833 and rs2273773, was linked to high blood pressure and body obesity. In a study of 3501 Pima Indians, who had a high incidence of Type 2 diabetes (44%) and two SIRT1 SNPs (rs10509291 and rs7896005), Type 2 diabetes was connected to Type 2 diabetes but not BMI (Shimoyama et al., 2011). Research subjects with the at-risk alleles in these SNPs had a reduced acute insulin response to glucose, which may raise the risk of Type 2 diabetes (Shimoyama et al., 2011).

Some SNPs are linked to leanness as well as an increase in obesity, suggesting that genetic variations in SIRT1 may protect against obesity and, in turn, protect against Type 2 diabetes. For instance, a study of two different Dutch groups found that the G allele of rs7895833 was associated with a lower BMI (Zillikens et al., 2009). The minor allele of these SNPs was more prevalent in the control lean group compared to the case population, suggesting that it may have a protective effect against obesity (Clark et al., 2012). Several SIRT1 SNPs were identified in a different study that was associated with obesity in morbidly obese adults. Even though individual SIRT1 SNPs may have a direct impact on BMI, there is evidence that these genetic differences may also have an impact on how well a treatment works. According to measurements of fasting plasma glucose, insulin sensitivity, and liver fat in a controlled lifestyle intervention study of Caucasian participants at risk for Type 2 diabetes, patients with the minor allele of SNP rs12413112 did not respond to the lifestyle interventions of increased exercise and reduced dietary fat as well as those with the wild-type allele (Weyrich et al., 2008). These findings suggest that studying SIRT1 genetic variants may help determine the type of medication that prediabetic or diabetic patients may respond to most favorably.

The human SIRT3 gene has also been shown to contain several SNPs and the effects that go along with them. The TT genotype of the SIRT3 G477T marker was found to increase survival in the elderly in 2003(Rose et al., 2003), although relationships with diabetes or obesity were not investigated. In a separate investigation, Dransfeld et al. discovered two SNPs in the functional region of the SIRT3 protein in 640 Caucasians, but they were unable to evaluate the functional significance of these polymorphisms. Hirschey et al. separately discovered and described the SNP rs11246020 (change of valine to isoleucine in the sirtuin conserved region of SIRT3) in a Caucasian population with fatty liver disease in 2011. They found that this polymorphism raises the possibility of acquiring metabolic syndrome. Because recombinantly expressedSIRT3 with this point mutation demonstrated decreased activity compared to the wild-type protein, it has been hypothesized that this reduction in SIRT3 function may predispose a patient to developing the metabolic syndrome. There is still much to learn about SIRT3 polymorphisms and their associated effects, and there is a critical need to find the most effective, individually tailored treatments for patients with metabolic syndrome or Type 2 diabetes.

Chapter 4

4.1. Targeting sirtuins for the treatment of diabetes

4.1.1. Resveratrol: A first generation of sirtuin activators

Significant research has gone into developing innovative therapies that can activate sirtuins because of the possibility that sirtuins could be used to effectively treat or perhaps prevent diabetes. The most extensively researched of these is a naturally occurring substance called resveratrol. In 1940, the white hellebore plantyielded resveratrol for the first time. To cure hyperlipidemia among other conditions, Nonomura et al (Liu et al., 2015). 1963 isolated resveratrol from root extracts used in conventional Japanese and Chinese medicine. Reducing hepatic cholesterol buildup in rats on a high-fat diet was demonstrated by resveratrol. When this drug was found in red wine, there was a prominent deal of interest in figuring out whether this substance was what gave red wine its health benefits and what caused the so-called French paradox, in which the French consume a lot of saturated fats but have one of the lowest rates of cardiovascular disease. On a search engine to find SIRT1activators in 2003, resveratrol came up first.

The efficacy of resveratrol to enhance mouse lifetime was examined in light of the relationship between sirtuins and lifespan. Resveratrol restored normal longevity to mice fed a high-calorie diet, even if treatment to animals did not increase lifespan. These early investigations shown that resveratrol reduced blood sugar and insulin levels, improved glucose tolerance, and boosted insulin sensitivity in mice fed a high-fat diet. It also decreased blood insulin levels and enhanced glucose tolerance (Pearson et al., 2008). Resveratrol effectively decreased blood glucose levels,

in animal models of diabetes brought on by the -cell toxin streptozotocin. In animals fed high-calorie diets, resveratrol also decreased hepatic fat buildup. As a result, evidence from rodent models supports the use of resveratrol as a diabetes therapy (Shang et al., 2008) (Poulsen et al., 2012) (Bujanda et al., 2008).

Even though oral resveratrol supplements are widely available, the findings supporting resveratrol's efficacy in people are not entirely understood. In a systematic review that compiled the opinions of specialists who attended the first International Conference on Resveratrol and Health (Resveratrol 2010), Vang et al. came to the conclusion that the evidence in humans is insufficient to support giving resveratrol to people as a disease treatment (Vang et al., n.d.). There is no experimental data offered; the authors only describe two clinical trials by Elliott et al. that demonstrated decreased fasting, postprandial, and postprandial insulin levels in Type 2 diabetes patients treated with resveratrol (*Resveratrol (SRT501): Development Halted | Science | AAAS*, n.d.). Vang et al. note that more clinical trials should be finished even with the scant human data currently available because the evidence in animal models are encouraging. Resveratrol has been the subject of more recent small-scale studies to see how it affects human diabetes.

Resveratrol treatment (75 mg/day for 12 weeks) showed no impact on insulin sensitivity in non-obese women with normal glucose tolerance, according to a research by Yoshino et al. (n = 15 in each group) (Yoshino et al., 2012). Reportedly, when Poulsen et al. treated obese but otherwise healthy males with resveratrol (500 mg, three times daily for 4 weeks; n = 12 in each group), there were no changes in body composition and insulin sensitivity (Poulsen et al., 2013). Timmers et al. performed a crossover study, similar to Poulsen et al., and found that resveratrol treatment (150 mg once daily for 29 days; n = 11), decreased circulating glucose, insulin, and

leptin, improved insulin sensitivity as assessed by the homeostatic model assessment of insulin resistance, mildly increased intramyocellular lipid levels, but increased hepatic lipids (Timmers et al., 2011). It is uncertain why there is a difference between the Poulsen et al. and Timmers et al. studies, however, it may be due to various study designs, resveratrol dosages, and the small number of participants studied. Recent investigations on glucose intolerant patients showed more effective effects for resveratrol than similar trials on fat, healthy individuals.

For instance, Brasnyo et al. administered resveratrol (5 mg, twice daily for 4 weeks) to 19 Type 2 diabetic males and discovered modest improvements in insulin sensitivity as measured by the homeostatic model evaluation of insulin resistance and by increased photophosphorylation of Akt in platelets (Crandall et al., 2012). Furthermore, giving resveratrol to older patients with poor glucose tolerance (1-2 g/day for 4 weeks; n = 10) did not affect their fasting glucose or insulin levels, but it did enhance their glucose tolerance and increase their sensitivity to insulin (Brasnyó et al., n.d.)

Reviewing the metabolic effects of resveratrol in many animal and human investigations, it is clear, resveratrol has no impact on metabolism in non-diabetic, non-obese subjects in rodent and human studies. This might be a benefit of resveratrol since a healthy metabolic state has a minimal risk of hypoglycemia. Additionally, there is a tendency for greater dosages of resveratrol to reduce body weight under situations of positive energy balance, for example high-fat meals or obesity. However, as moderate gastrointestinal symptoms including nausea and diarrhea have been reported at high doses in clinical research, this may be an indirect consequence of resveratrol (Brown et al., n.d.). Interestingly, the evidence strongly suggests that resveratrol has a positive impact on lowering blood sugar levels and/or improving insulin

sensitivity in subjects who are more metabolically stressed, such as mice who were given a high-fat diet or humans with confirmed diabetes diseases.

While these results are encouraging, additional clinical information from larger studies is unquestionably required to establish the proper dosage and evaluate the long-term safety and effectiveness of utilizing resveratrol to treat diabetes. For this purpose, the effects of resveratrol on diabetic conditions are the subject of more than 20 research that are now listed on the clinical trials database supplied by the US NIH.

According to promising research on resveratrol, treating diabetes by activating sirtuins may be a possibility. Although long-term trials have not been completed, it is hopeful since resveratrol is great tolerated in patients along with few adverse effects except minor gastrointestinal problems (nausea and diarrhea) at high doses (Brown et al., n.d.). Importantly, very few occurrences of hypoglycemia have been associated with resveratrol therapy, even in animals or people whose blood sugar levels were normal previous to treatment. Although encouraging, many of resveratrol's benefits in decreasing blood sugar and insulin sensitivity in people have been limited. This may be because unmodified resveratrol given orally in people has a low absorption (Walle et al., 2004) (Goldberg et al., 2003). Furthermore, whether resveratrol activates SIRT1 directly or indirectly has recently come under scrutiny. Resveratrol has been demonstrated in numerous studies to activate SIRT1 and produce anti-diabetic benefits, regardless of the molecular processes involved. However, resveratrol may have several non-specific effects due to indirect SIRT1 activation. This emphasizes, even more, the requirement for next-generation sirtuin activators that are more focused and efficient than resveratrol.

4.1.2. The next generation of sirtuin activators

More potent sirtuin activators are being developed right now. AS101, a tellurium compound created at Bar-Ilan University, has recently been revealed to boost SIRT1 protein expression and activity and has potential as an anti-diabetic medication. Administration of AS101 before clinical hyperglycemia reduced blood glucose levels and improved insulin sensitivity in a rat model of type 2 diabetes, and administration of AS101 after the onset of type 2 diabetes partially reversed normal glucose homeostasis (Huynh et al., 2013).

As the top pharmaceutical company investigating the discovery of small compounds that activate sirtuins, Sirtris, a GlaxoSmithKline company (MA, USA), has another group that is especially focused on creating small molecule medications that target sirtuins to cure diseases of aging. A report on the anti-diabetic properties of SRT1720, a small-molecule SIRT1 activator that is structurally distinct from resveratrol but 1000 times more effective, was released by Sirtris in 2007. Initial research suggested that SRT1720 treatment for diet-induced obese mice lowered fed glucose levels over the course of 10 weeks, with findings similar to those seen in a genetic mouse model of Type 2 diabetes (Huynh et al., 2013), suggesting the possibility of treating Type 2 diabetes using SIRT1 activators.

Additional research revealed that SRT1720 induces fatty acid oxidation to protect against diet-induced obesity and insulin resistance. As a result of decreased liver steatosis, greater insulin sensitivity, improved locomotor activity, and normalized gene expression profiles and markers of inflammation and apoptosis, Minor et al. recently reported that obese mice treated with SRT1720 lived longer. While three other SIRT1 small molecule activators created by Sirtris (SRT2104, SRT2379, and SRT3025) are now completing clinical trials, SRT1720 is still in the preclinical

testing stage due to disagreement around off-target effects. SRT2104 oral dose in healthy volunteers was tested for tolerability and pharmacokinetics in Phase I clinical trials; the results, which were recently published, showed no adverse reactions (Huynh et al., 2013). Hoffman et al. cited unpublished results in this work that suggest SRT2104 treatment improved glucose and insulin homeostasis in diet-induced obese mice and genetically obese ob/ob mice. Clinical study findings showed that SRT2104 was well tolerated at doses up to 3.0 g, however, adequate absorption was hampered by the tiny molecule's insolubility. Phase II clinical trials of SRT2104 are required to demonstrate the effectiveness of SRT2104 as a SIRT1 activator in humans, despite even though adverse effects were identified in the research mentioned.

The supplementation of NAD+ precursors is another potential therapeutic approach for the activation of sirtuins, in addition to the pharmacological candidates mentioned above. Exogenous nicotinamide riboside, a vitamin precursor to NAD+, was found to increase NAD+ and activate Sir2 in 2007, extending the lifespan of yeast (Belenky et al., 2007). This result is significant since it was obtained without calorie restriction, indicating that NAD+ precursors may directly activate mammalian sirtuins. In fact, Mice nicotinamide riboside showed increased levels of NAD+ in tissues, a small improvement in glucose tolerance, and a decrease in insulin secretion (Cantó et al., 2012). Additionally, supplementation with nicotinamide mononucleotide, another NAD+ precursor, showed the ability of these substances to activate sirtuins. Treatment with nicotinamide mononucleotide reversed the high-fat diet-induced depletion of NAD+ levels and enhanced glucose tolerance in a mouse model of Type 2 diabetes, which was partly attributable to the activation of SIRT1(Yoshino et al., 2011). NAD+ precursors may be promising new sirtuin activators to manage and treat Type 2 diabetes based on the improvement in glucose tolerance seen in these two investigations.

Because SIRT1 is the most well-understood sirtuin, the therapeutic techniques to activate sirtuins mentioned above are mostly focused on activating SIRT1. There are still a few methods that need to be researched that could activate SIRT1, SIRT3, and/or SIRT6. Since SIRT3 and SIRT6 are also NAD+-dependent enzymes, it is important to investigate the activity of these sirtuins when given NAD+ precursors as a potential treatment for metabolic disorders (Huynh et al., 2013). Additionally, several targets of activation will undoubtedly move to the front of attempts to find novel therapies as the mechanisms of sirtuin regulation become well known. For instance, sirtuins may be precisely activated by targeting transcriptional regulators or mechanisms of post-translational changes of the sirtuins.

Chapter 5

Conclusion

The evidence for sirtuins and their anti-diabetic potential is convincing, and ongoing research will establish if sirtuin activators will be a viable diabetes treatment. While sirtuins do not appear to enhance human lifespan as originally envisaged, it is becoming obvious that sirtuins may improve quality of life and extend healthspan by combating age-related problems such as the metabolic abnormalities associated with Type 2 diabetes. Human SNP studies and high-fat fed animal models show that sirtuin malfunction may be a biological pathway linking overnutrition and diabetes. Thus, targeting sirtuins may be a useful approach of treating or even preventing diabetes caused by aging or an unhealthy lifestyle.

Future perspective

More than 30 clinical trials are under underway to investigate the safety and efficacy of resveratrol and other SIRT1 activators in the treatment of diabetes. While drug discovery for SIRT3 and SIRT6 activators is still in its early stages, the evaluation of animal models given here underlines the therapeutic promise of SIRT3 and SIRT6 activators in treating the metabolic abnormalities associated with Type 2 diabetes.Because SIRT1 and SIRT3 levels decrease with aging and high-fat feeding, decreased SIRT1 and SIRT3 activity is linked to metabolic abnormalities consistent with Type 2 diabetes, and human SNPs that result in decreased sirtuin activity are linked to the development of metabolic syndrome, it is tempting to speculate that sirtuin activity may be a mechanistic link between aging and overnutrition and Type 2 diabetes. If this is accurate, sirtuin activators could be useful diabetes treatments.While it is too early to

tell, medications that prevent the loss in sirtuin activity caused by age or overnutrition could offset the continuous rise in diabetes incidence and death.

Chapter 6

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