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The effect of a putative acyl-CoA synthetase 5 inhibitor on lipid accumulation and insulin release from clonal pancreatic beta-cell

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BOSTON UNIVERSITY
SCHOOL OF MEDICINE

Thesis

**THE EFFECT OF A PUTATIVE ACYL-COA SYNTHETASE 5 INHIBITOR ON
LIPID ACCUMULATION AND INSULIN RELEASE FROM CLONAL
PANCREATIC BETA-CELL**

by

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DEDICATION

I would like to dedicate this work to my parents and my grandma, who has always been supportive for me in ups and downs. To my grandad, whose spirits encouraged me in pursuing the question I want to find answers for.

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ABSTRACT

It is estimated by the World Health Organization (WHO) that 422 million people had diabetes worldwide in 2014, including 30.3 million people in the US. The cost of treating the disease is has tripled from 2003-2013 due to the increased number of patients. One of the genes strongly associated with type 2 diabetes (T2D) is the transcription factor 7 like 2 (TCF7L2). A single nucleotide polymorphism (SNP) of the TCF7L2 results in increased expression of long chain acyl-CoA synthetase 5 (ACSL5) while deletion of this part of the TCF7L2 gene reduces ACSL5 mRNA level. The regulation of ACSL5 gene expression by the high risk TCF7L2 allele highlights the importance of investigating the role of ACSL5 in T2D. ACSL5 is one of a family of enzymes that activates FA to its CoA ester and is required for FA metabolism within cells. Mice lacking this protein have reduced fat mass and are more insulin sensitive.

Chronic exposure of clonal pancreatic β -cells to excess nutrients has been shown to result in increased intrinsic lipid droplets, reduced insulin content, a left-shift in glucose dose-dependent insulin secretion curve characterized by basal insulin hypersecretion (IH) and blunted glucose stimulated insulin secretion (GSIS). We tested the hypothesis that the use of a putative ACSL5 inhibitor (Adipo C) can reduce accumulated lipid droplets, rescue insulin content and reverse the left-shift in glucose dose-dependent insulin secretion curve.

INS-1 (823/13) cells were cultured in either 4 mM or 11 mM glucose media representing physiological and excess nutrients environment. Adipo C (10-25 μ M) was added to cells to both acutely (2 hrs) and chronically (72 hrs) inhibit ACSL5 activity. Thin layer chromatography with C11 Bodipy fatty acid (BFA) was used to detect acute fatty acid incorporation into neutral lipids. Nile red was used to visualize intrinsic lipid droplets inside cells. Intracellular Ca^{2+} activity was detected using fura 2. Insulin assay was measured by HTRF.

Acute fatty acid incorporation and lipid accumulation were reduced in cells exposed to Adipo C. An Adipo C concentration dependent right shift of glucose dose-dependent insulin release and increased insulin content were observed. 11 mM glucose cells cultured in 25 μ M Adipo C showed decreased intracellular Ca^{2+} activity at 3 mM glucose and increased Ca^{2+} activity at 12 mM glucose, which are characteristic of cells cultured in 4 mM glucose having reduced lipid stores. These results all indicate possible protective effects on β -cells exposed to excess nutrients. Islets of T2D patients who have a physiologically elevated blood glucose level are exposed to a similar excess nutrient environment. Therefore, the results illustrated here warrant further research on Adipo C compound to explore its therapeutic potential on T2D.

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LIST OF ABBREVIATIONS

ACSL5	Long-chain CoA synthetase 5
α -GP	α -glycerophosphate
BFA	Bodipy fatty acid
BSA	Bovine serum albumin
CE	Cholesteryl ester
CPT-1	Carnitine palmitoyl-transferase 1
CVD	Cardiovascular disease
DAG	Digacylglycerol
DZ	Diazoxide
DMSO	Dimethyl sulfoxide
ESRD	End-stage renal disease
GLP	Glucagon-like peptide
GLT	Glucolipototoxicity
GSIS	Glucose stimulated insulin secretion
IDF	International Diabetes Federation
IFT	Impaired fasting glycemia
IGT	Impaired glucose tolerance
IH	Insulin Hypersecretion
LC-CoA	Long-chain CoA
LC-FA	Long chain fatty acid
PFK-1	Phosphofructokinase-1

PKC.....	Protein Kinase C
PIP2.....	Phosphatidyl-myo-inositol 4,5-bis-phosphate
PLC.....	Phospholipase C
SE.....	Standard error
SNP.....	Single nucleotide polymorphism
T2D.....	Type 2 diabetes
TCF7L2.....	Transcription factor 7 like 2
TG.....	Triglyceride
TLC.....	Thin layer chromatography
WHO.....	World Health Organization

Introduction

Diabetes and its economic impact

Diabetes is a chronic disease that occurs when the pancreas is incapable of producing enough insulin to regulate blood glucose level or when the body fails to respond to insulin (1). Type 1 diabetes, also known as insulin-dependent or childhood-onset diabetes, is characterized by deficiency in insulin production and it accounts for less than 10% of total diabetes. The cause of type 1 is thought to be destruction of β -cells due to autoimmune disease (2). Type 2 diabetes, formerly referred to as non-insulin-dependent diabetes, accounts for the vast majority of diabetes (1). It is estimated that 30.3 million people, 9.4% of the U.S. population, had diabetes in 2015, including type 1 and 2 (3). World Health Organization (WHO) report estimated that around 422 million adults, 300 million more compared to 1980, had diabetes worldwide in 2014. Prevalence of diabetes globally has doubled in the past 3 decades, increasing from 4.7% to 8.5% (2). The substantial increase of the global prevalence in diabetes mirrored the similar trend in the rate of people who are obese or overweight (2).

Diabetes imposes large economic burden on society via medical costs and indirect costs related to lose of productivity, increased mortality and so on. Direct medical costs involve the expenditure of outpatient and inpatient care for treating and preventing diabetes, medication and medical devices, and long-term care. It is estimated, by the International Diabetes Federation (IDF), that the total health-care expenditure on diabetes globally has tripled from 2003-2013 due to the increased number of diabetic patients and

per capita spending on diabetes (4). Also, the prevalence of diabetes has risen faster in developing countries than in developed countries (2).

Classification of diabetes

Diabetes mellitus is defined as a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism caused by defects in insulin secretion, insulin action, or both (1).

Type 1 refers to the processes of β -cell destruction that results in an “insulin required for survival” diabetes mellitus. Type 1 is usually characterized by the presence of anti-islet cell or insulin antibodies which are the indicators of autoimmune processes that lead to beta-cell destruction (5).

Type 2 is characterized by disorders of insulin secretion and insulin action, both of which usually present at the same time. People with type 2 diabetes are frequently insulin resistant, which means resistant to the action of insulin. This form of diabetes can go on undiagnosed for years because hyperglycemia can often go unnoticed and does not provoke symptoms in early stages. There are other specific types of diabetes caused by less common causes (5).

It is no longer recommended to use the term “insulin-dependent diabetes mellitus” and “non-insulin-dependent diabetes mellitus” because it classifies patients on the basis of treatment instead of pathogenic reasons (5). In clinical staging, diabetes mellitus can be subdivided into: Insulin requiring for survival, Insulin requiring for metabolic control, Not insulin requiring. (5)

Diagnosis criteria and complications of diabetes

The diagnostic point for diabetes is fasting plasma glucose ≥ 7.0 mmol/L, selected based on micro-vascular complication. However, the risk for macro-vascular disease, such as heart attack or stroke, increases even before the set diagnostic point. Impaired glucose tolerance (IGT) and Impaired fasting glycemia (IFG) are referred to as intermediate states between normal glucose homeostasis and diabetes. What should be noted is that IGT refers to abnormalities of glucose regulation in post prandial condition whereas IFG refers to impaired glucose regulation in the fasting state (5).

Diabetes mellitus may present symptoms such as thirst, polyuria, blurring vision and weight loss. Consequences of these pathogenic processes include the wear-out of the β -cells of the pancreas, insulin deficiency and insulin resistance. Uncontrolled diabetes can lead to severe eyesight impairment or blindness, kidney failure, and lower limb amputation. These long-term consequences significantly lower the life quality of diabetes patients. Diabetic retinopathy is responsible for 2.6% of blindness worldwide in 2010 (6). Data from 54 countries demonstrates that 80% of end-stage renal disease (ESRD) results from diabetes, hypertension or a combination of the two (7). Individuals with diabetes historically have a two or three times higher rate in cardiovascular disease (CVD) compared to non-diabetics (8). Diabetes increases the rate of amputation 10-20 times because of infected, non-healing foot ulcers (9).

Excess body fat and physical inactivity are the strongest risk factors for type 2 diabetes. Regular physical activity is shown to effectively reduce blood glucose and is an important contributor to control weight, and prevent overweight or obesity, all of which

are linked with higher diabetes prevalence (10). Therefore, the more prevalent physical inactivity worldwide is of increasing concern. Specifically, physical inactivity among adolescents is alarmingly high, with 84% of girls and 78% of boys falling under the minimum standards of physical activity. Similar with physical inactivity, being overweight or obese is also strongly associated with diabetes. According to a global estimate, more than one in three adults aged over 18 years was overweight and more than one in 10 was obese (2). Higher waist circumference and higher body mass index (BMI) are strongly associated with an increase in risk of type 2 diabetes (11).

Molecular Mechanism of Type II Diabetes

Molecular Mechanism of Insulin Secretion

The function of β -cells depends on their capability to sense blood glucose changes in the environment (12). The well-acknowledged pathway of glucose stimulated insulin secretion (GSIS) involves two parts: triggering and amplification (13). Elevated blood glucose increases glucose influx into the β -cell through Glut-2 which quickly equilibrates glucose across membrane. Glucose metabolism was increased because of high sensitivity (high K_m) of glucokinase to glucose, and that leads to elevated ATP/ADP ratio, which results in closure of ATP-sensitive K^+ (K_{ATP}) channels and subsequently depolarizes the cell membrane. Voltage-gated Ca^{2+} channels open in response to the depolarization of the plasma membrane. A rise in intracellular Ca^{2+} is a prerequisite for exocytosis of insulin granules from the immediately releasable pool (14).

The amplification pathway of insulin release has been suggested to involve a number of factors generated from glucose and fatty acid metabolism (14) (15), some of which are described below. Long chain acyl-CoA (LC-CoA) is a major source of energy for pancreatic β -cells under physiological glucose levels (16). Increased glucose metabolism induces a rise in malonyl-CoA, which is known to inhibit carnitine palmitoyl-transferase I (CPT I) activity and therefore fatty acid oxidation. As a consequence, the LC-CoA ester level would rise in the cytoplasm providing increased availability to form signaling molecules in β -cells (15). LC-CoA stimulated fusion of secretory granules to the cytoplasmic membrane thereby amplifying secretion (17). It is also observed in β -cells that increased malonyl-CoA levels were correlated with elevated *de novo* lipid synthesis and diacylglycerol (DAG). DAG is a known activator of protein kinase C (PKC), the activation of which is known to stimulate insulin release (18). In addition, α -glycerophosphate (α -GP), which is a glucose metabolite derived from glycolysis, also participates in the synthesis of DAG. Therefore, malonyl-CoA and α -GP are important regulators of amplifying pathways of insulin release. Metabolic signals activate phospholipase C (PLC) through an elevated cytosolic Ca^{2+} level and possible other pathways. PLC then hydrolyzes phosphatidyl-*myo*-inositol 4,5-bis-phosphate (PIP₂) and produces inositol 1,4,5-P₃ (IP₃), whose main function is to amplify the Ca^{2+} signal, and DAG, which serves multiple purposes as a second messenger to phosphorylate proteins through activation of protein kinase C (PKC) (14) (18), and to directly stimulate exocytosis by binding to Munc13-1 (19).

Oscillatory Pattern of Ca^{2+} and Insulin Secretion

Oscillations in insulin secretion have been shown to exist in perfused clonal insulin-secreting-cell lines, dissociated islets and intact islets (20) (21). The importance of this oscillatory pattern in insulin release is highlighted by the fact that it is often impaired or defective in patients with type 2 diabetes (22). A generally accepted model proposed that oscillations in glucose metabolism are implicated in Ca^{2+} oscillations and drive oscillations in insulin release. After glucose enters β -cells through the Glut-2 transporter, it is phosphorylated by glucokinase, which is known as the glucose sensor since it regulates glucose influx by sensing the physiological concentration of glucose (23). In addition to phosphorylation by glucokinase, glucose influx is furthered by phosphofructokinase-1 (PFK-1). PFK-1 is allosterically regulated. It is activated by AMP and fructose 1, 6-biphosphate and inhibited by ATP. The mechanism of PFK-1 being activated by its product provides exquisite sensitivity to the energy state of the cell (24). The autocatalytic activity of PFK-1 results in bursts of ATP production from reactions downstream of glycolysis and citric acid cycle, which in turn inhibits PFK-1 activity and causes oscillation of ATP/ADP ratio (25). The oscillating ATP/ADP ratio is implicated in closing and reopening of K_{ATP} channels, which subsequently causes depolarization of the plasma membrane, Ca^{2+} influx (13) and insulin release in an oscillatory pattern (26) (15). But oscillations in insulin release can be independent of Ca^{2+} as demonstrated when islets were perfused with 100 μM diazoxide and 30 mM KCl to maintain opening of K_{ATP} channels and high intracellular Ca^{2+} concentration (20).

Proinsulin biosynthesis and Insulin Secretion

In addition to insulin secretion, the function of beta-cells also includes production and storage of insulin. Normally, insulin stores and a readily available insulin pool for secretion are maintained by proinsulin biosynthesis (27). In general, nutrients that stimulate insulin secretion also promote proinsulin biosynthesis. Glucose can serve as a signal to both stimulate glucose induced insulin secretion (GSIS) and proinsulin biosynthesis. Even though many nutrients can regulate insulin secretion and proinsulin biosynthesis simultaneously, there must be a divergent point of these two functions since the cell apparatus and metabolic pathways involved in these two functions are distinct. Prolonged exposure to long-chain fatty acid, especially saturated fatty acid such as palmitate has been shown to potentiate GSIS but at the same time inhibit glucose-induced proinsulin biosynthesis (28) (29). Palmitate can participate in ceramide synthesis as substrate and ceramide has been shown to decrease proinsulin biosynthesis (30) through extracellular signal-regulated kinases (ERKs) pathway (31)

Hyperinsulinemia, Insulin Resistance, and Glucolipototoxicity,

Hyperinsulinemia (HI) refers to an increased plasma insulin level at basal glucose concentration. Obesity is shown to be associated with a significant elevation in insulin secretion at both fasting and post-prandial conditions even when glucose tolerance is normal (32). Insulin resistance is tightly linked to hyperinsulinemia by definition and there are different theories in explaining this causal relationship. The elevated insulin level has been seen as a compensatory response when an individual is systemically insulin resistant (33). Alternatively, it has been proposed that HI due to chronic exposure to excess nutrients

may be partly responsible for insulin resistance rather than just the consequence of it (34). HI has been shown to induce insulin resistance by increasing lipid accumulation in peripheral tissue, and down-regulating insulin receptors, namely in hepatocytes (35). Also, moderate disruption in insulin secretion using streptozotocin can slow weight gain in B6 mice on a high fat diet and partially prevent developing insulin resistance. (35)

Built on the close association of hyperinsulinemia with obesity and the fact that GSIS is triggered by glucose influx and possibly amplified by lipids, fatty acids and its derivatives, increasing interest has been shown in investigating the concepts of glucotoxicity (chronically elevated glucose) (36), lipotoxicity (37), and both, referred to as glucolipotoxicity (GLT) (38) to explain the abnormal insulin secretion in T2D. GLT results from the combined, toxic effects of elevated glucose and fatty acid on pancreatic beta-cell function and survival (38). GLT has been shown to reduce GSIS and inhibit proinsulin biosynthesis (39)(40)(41). In contrast, GLT increases basal insulin secretion from the β -cell and causes basal hyperinsulinemia (42).

The model in figure 1 includes the causal relationship between insulin resistance and hyperinsulinemia. GLT caused by excess nutrients can lead to insulin resistance (IR) in peripheral tissues such as muscle. The resulting condition of hyperglycemia causes the β -cell to compensate by increasing insulin release and leads to hyperinsulinemia (HI) (shown by green arrows). The left side of the figure predicts that the effect of GLT to induce β -cell basal hyper insulin secretion (IH) can promote basal HI which can in turn exacerbate insulin resistance (shown by red arrows).

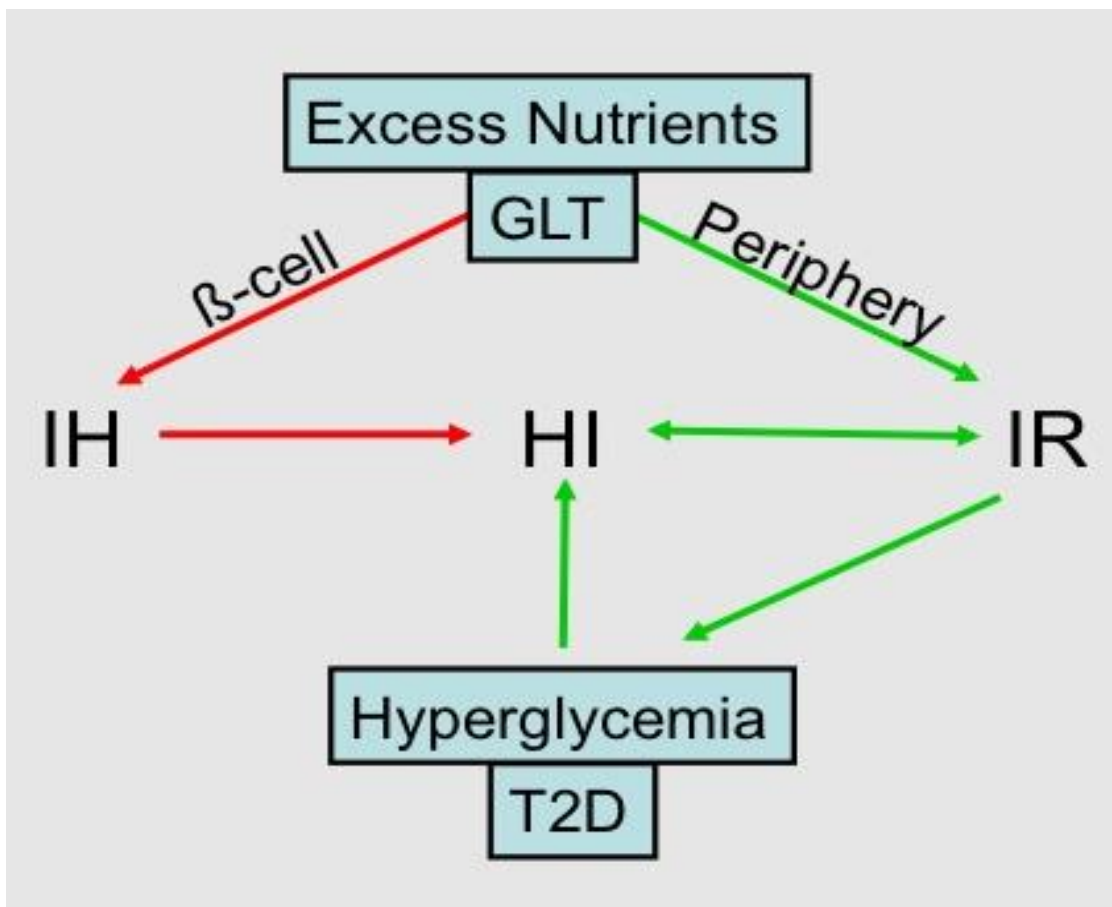


Figure 1. Model illustrating the contributions of insulin hypersecretion (IH) and hyperinsulinemia (HI) in the development of insulin resistance (IR) under glucolipotoxic (GLT) environment.

Long-chain acyl-CoA synthetase (ACSL)

Long-chain Acyl-CoA synthetase (ACSL and ACS are used interchangeably) (43) is responsible for the conversion of long-chain fatty acid (LC-FA) and coenzyme A (CoA) to LC-CoA (44). LC-CoA can consequently enter pathways of β -oxidation, desaturation, elongation, lipid synthesis including triglyceride (TG) synthesis, cholesteryl ester (CE) synthesis and signaling molecules such as diacylglycerol (DAG) and ceramide (45).

Five ACSL isoforms (ACSL1-5) were cloned from rats (46)(47)(48). ACSL isozymes have specific tissues and organelle distribution. Among them, ACSL5, having a molecular mass of 70 kDa, is highly expressed in intestine and also present in liver (46). Also, ACSL5 is the only isoform that is located on the mitochondrial outer membrane (46). For comparison, ACSL1 is a membrane protein in endoplasmic reticulum (ER) (49) whereas ACSL4 is primarily found in mitochondrial associated membrane (MAM) (43). These different locations indicate that each ACSL isoenzyme could work independently and be responsible for different pathways.

Triacsin C is a known ACSL inhibitor, can be used to block the conversion of fatty acids to LC-CoA. Triacsin C has been used to acutely affect lipid metabolism in INS-1 cells and the authors found no obvious effects on glycolytic flux or GSIS (51). Use of inhibitors of ACSL has also suggested the existence of different acyl-CoA pools inside cells, as these inhibitors are capable of inhibiting only part of the acyl-CoA related pathways (49).

All isoforms of ACSL belong to a luciferase superfamily because they contain two luciferase-like structures and a linker to connect them. An AMP-binding site and a predicted fatty acid binding site locate in the two luciferase-like regions (48). Notably, ACSL1, ACSL2, and ACSL5 share more than 60% similar structure and amino acids. Despite the high degree of resemblance, the ACSL1 and ACSL5 mRNA expression is regulated independently. In liver, ACSL1 mRNA level was elevated after 24 hr fasting (52). In the intestine ACSL5 mRNA was unchanged after different diet in mice, but that in liver was significantly decreased by fasting (46) and increased by refed after 24 hr fasting (52).

The specific role for ACSL5 in cells has yet to be completely established. ACSL5 has a broad substrate specificity for saturated (12-18 carbons long) and unsaturated fatty acids (16-20 carbon long) (46). ACSL5 mRNA does not change during adipocyte differentiation (53). The high level expression of ACSL5 mRNA in intestine epithelial cells suggests the role of uptake of dietary fatty acid (46). Its mRNA level was also increased with a rise in glucose dose in culture media and in the presence of insulin in primary hepatocyte (52). Overexpression of ACSL5 in rat hepatoma is associated with higher acyl-CoA synthetase activities and increased rate of *de novo* synthesis of TG and re-acylation pathways of synthesizing TG without increasing the metabolism of fatty acids (54). It has also been recently reported that ACSL 5 expression ablated mice had increased metabolic rates and insulin sensitivity, along with delayed triglyceride absorption leading to decreased fat mass (55).

The transcription factor 7 like 2 (TCF7L2) gene and ACSL5

The gene encoding transcription factor 7 like 2 (TCF7L2) is reported to be one of the genes consistently and strongly associated with type 2 diabetes in Genome-wide association studies (GWASs) (56) (57). However, genes reported by GWASs are linked to a given trait but sometimes not the precise culprit gene. Many studies have been conducted to understand the effect of TCF7L2 in the pathogenesis of type 2 diabetes mellitus however a clear role for this gene has not been established. Therefore, it is still not clear that TCF7L2 is actually the culprit gene affecting diabetes. Studies on the role of TCF7L2 have concentrated on several tissues including pancreatic islets (58), liver (59), adipose tissue

(60) and intestinal cells (61). Among them, studies on intestinal cells may be the most plausible since the TCF7L2 gene and β -catenin, its binding partner, mediate proglucagon gene regulation. Proglucagon is cleaved into glucagon-like peptide (GLP-1) in the intestinal tract (62). GLP-1, produced by enteroendocrine L cells in response to food intake (63), is an incretin and accounts for 50-70% of post prandial insulin secretion (64). This response initiated by GLP-1 is impaired due to reduced GLP-1 concentration in type 2 diabetes patients (65). Furthermore, brain GLP-1 mediates glucose and food intake homeostasis, possibly through repressed AMPK activity (66).

The present consensus as a result of fin3 mapping is that the T allele of rs7903146 within TCF7L2 is the causal variant that is associated with type 2 diabetes (67)(68)(69). Following this direction, using genome editing, microarrays and chromatin conformation capture it has recently been shown that the ACSL5 gene is located in the same topologically associating domain as TCF7L2 (62). ACSL5 mRNA is consistently reduced when conducting additional deletion of 66-104 base pairs containing rs7903146. In fact, the deletion of this SNP region in a human intestinal related cell line abolished chromatin contacts with the ACSL5 promoter and these two regions were conserved in human colon tissues (62).

Adipo C putative ACSL5 inhibitor

After screening compound libraries the Adipogenics Co. discovered a particular compound that was effective in reducing FA incorporation into lipid of cultured human differentiated preadipocytes. Adipogenics went on to further characterize this inhibitor

both in cell culture of differentiated preadipocytes and in mice. The compound referred to here as Adipo C, was shown to dose dependently inhibit fluorescent Bodipy FA uptake into differentiated preadipocytes and its subsequent incorporation into intracellular lipids. The compound inhibited ACSL activity in cell extracts and specifically reduced the level of ACSL5 in these cells as shown by western blot of ACSL isoforms. The compound also reduced weight gain in mice on a high fat diet.

In this thesis we have attempted to characterize this putative ACSL5 inhibitor in clonal pancreatic β -cells (INS-1) and to determine its effects on lipid accumulation, intracellular Ca^{2+} , insulin content and secretion.

METHODS

INS-1 cell culture

INS-1 cells (832/13) (31) were cultured in RPMI 1640 containing: 11 mM glucose, 10% Fetal Bovine Serum (FBS), 50 IU/ml penicillin and 50µg/ml streptomycin, 10mM Hepes, 1 mM pyruvate, 2 mM L-glutamine and 50 µM β-mercaptoethanol. In addition, glucose was added to RPMI without glucose to achieve 4 mM glucose RPMI 1640 and used with the same additives. Cells were cultured at 37° C in humidified atmosphere (5% CO₂ -95% air). INS-1 cells were used between passages 60-80.

Adipo C compound

Adipo C is the name we use for the putative ACSL5 inhibitor discovered by the Adipogenics Co. Adipo C compound is reconstituted in dimethyl sulfoxide (DMSO) to 10 mM and 25 mM concentration and stored as aliquots at -20° C. The compound is diluted 1000X in media to achieve 10 µM and 25 µM for culture.

BODIPY fatty acid (BFA) preparation

BFA was purchased from Invitrogen (Molecular Probes) as an 11 carbon chain length with a terminal Bodipy moiety (C11). 1 mg was dissolved in 20 µL DMSO and complexed to 6.7 % bovine serum albumin (BSA) at 50° C to a 2 mM concentration of a 6:1 BFA to BSA molar ratio. DMSO was equal to or less than 0.1% when added to culture.

BFA incorporation and thin layer chromatography (TLC)

INS-1 cells cultured in 11mM Glucose RPMI 1640 were preincubated with 2 mM glucose media for 2hrs and then in Krebs buffer (containing 110 mM NaCl, 4.6 mM KCl, 5 mM NaHCO₃, 2 mM CaCl₂, 1 mM MgSO₄, 0.15 mM Na₂HPO₄, 0.4 mM KH₂PO₄, 20 mM HEPES, 0.05% BSA, pH 7.4) with Adipo C compound or DMSO for 30 mins. Cells were changed to test solutions containing 100 μM BFA and incubated 2 hrs at 37° C to measure BFA incorporation into neutral lipids. Test solutions included Krebs buffer containing 1 and 12 mM glucose with or without 10 μM Adipo C compound. At the end of 2 hrs methanol was added to wells to fix cells, which were scraped out and transferred to a glass tube. Chloroform was added to achieve a 2:1 chloroform:methanol (CM) ratio for lipid extraction. Lipids were then dried and stored at -20° C prior to lipid separation. Dried lipid was solubilized using CM and applied to Silica coated glass TLC plates (Whatman), which were oven dried at 100° C for 30 mins. Lipids were separated using a mobile phase composed of: hexane: diethyl ether: acetic acid (60: 38.5: 1.5). Epifluorescence from BFA was detected using 460 nm excitation and >515 nm emission wavelengths using a gel imager (GE Healthcare Life Science).

Nile Red

Nile red (Sigma) was reconstituted in DMSO (1 mg/ml) and added to cells at a 1:1000 dilution in 2 mM glucose RPMI media to a final concentration of 1 μg/ml. After 15 mins incubation Nile red was removed and cells were incubated in 2mM glucose RPMI. Pictures were taken using a Nikon TE200 microscope with excitation and emission wavelengths of 488 and 515 nm and 20X magnification.

Insulin Secretion

INS-1 cells were cultured in 96-well plates (Corning, Corning, NY) to an approximate density of 100,000 cells/well and incubated for 72 hrs with or without Adipo C (10-25 μ M). INS-1 cells were preincubated with RPMI containing 2mM glucose without serum for 2 hrs in the culture incubator. After 2 hrs cells were preincubated in 1mM glucose Krebs at 37° C for 30 mins. Test solutions were then added and incubated in a 37° C water bath for 2 hrs. Samples were then collected for insulin assay. For insulin content, cells were first detached using trypsin and counted using a hemocytometer, lysed in cold PBS containing 0.1% Triton X-100 (Sigma) and 25 mM NaOH and diluted in the same volume of 1% BSA Krebs buffer for insulin assay.

Insulin Assay

Insulin was measured in 1536 well plates using the HTRF insulin assay (Cis-Bio, Bedford, MA) using a Tecan M1000 Pro fluorescence plate reader.

Ca²⁺ Measurement

Intracellular Ca²⁺ in INS-1 cells was measured using fura-2 AM (Invitrogen, Carlsbad, CA). INS-1 cells were cultured in glass bottom 35 mm dishes (MAtek, Ashland, MA). The glass bottom dishes were coated with poly-D-lysine to allow better cell attachment. Cells were treated with Adipo C as described above for insulin secretion. INS-1 cells were preincubated with 2mM glucose RPMI for 1 hr prior to fura loading. 2 μ M

Fura 2 AM was loaded into cells in 2mM glucose RPMI with 0.1% pluronic acid for 30 mins. Fura 2 AM was removed and cells incubated for an additional 15 min for cleavage of the dye trapping free fura inside the cells. Imaging was performed on an Olympus DSU spinning disk confocal microscope at 37° C and 20X magnification. Fluorescence images were captured every 15 sec using wavelengths of 340 and 380 nm dual excitation and 510 nm emission. Data were analyzed using Nikon Sensing software.

Formation of INS-1 cell spheres and measurement of insulin oscillations

15 million INS-1 cells were plated in non-cell culture treated 60 mm dishes in RPMI. After 24 hrs cell colonies were divide into separate dishes with or without 25 μ M Adipo C to continue forming spheres 3 days before the experiment. On day of the experiment separated spheres were collected and pre-inbubated in 2 mM glucose RPMI for 2 hrs. Spheres were then loaded onto columns of cytodex beads (Sigma) maintained in a 37° C chamber and perfused with 1 mM glucose Krebs buffer solution for 30 min prior to collecting fractions at 15 sec intervals for 72 mins. Cells were perfused with 1 mM glucose (20 mins), 3 mM glucose (20mins), 12mM glucose (20mins) and 12 mM glucose + 30 mM KCl (12 mins). Insulin secretion was measured and analyzed as described above.

Statistical Analysis

The data are calculated as the mean of independent experiments and error bars are calculated as standard error (SE). Statistical significance was calculated by two-tailed

Student's t test or paired t-test where indicated. A p value of < 0.05 indicates significant change as indicated in figure legends.

RESULTS

Adipo C inhibits BFA incorporation into lipid

C11 BFA was used to track LC-CoA incorporation into lipids including triglyceride (TG), diglyceride (DG), and monoglyceride (MG). A representative TLC separation of lipid extracts from INS-1 cells incubated in 1 and 12 mM glucose with C11 BFA and with or without acute exposure to 10 μ M Adipo C for 2 hrs is shown in Figure 2. Lipid extracts from cells incubated in 12 mM glucose without Adipo C exposure showed increased incorporation of BFA into all three types of lipids (MG, DG, TG) compared to that from cells incubated in 1 mM glucose (compare lane 2 to 1). Adipo C treated cells showed less incorporation of BFA when glucose was raised from 1 to 12 mM (compare lane 4 to 3). In addition it appeared that Adipo C reduced basal incorporation of BFA into DG (compare lane 3 to 1). When C16 BFA was used instead of C11 BFA no difference in MG and TG incorporation was observed between control and Adipo C exposed group (data not shown). Adipo C exposure decreased some of the DG lipid levels stimulated by high glucose.

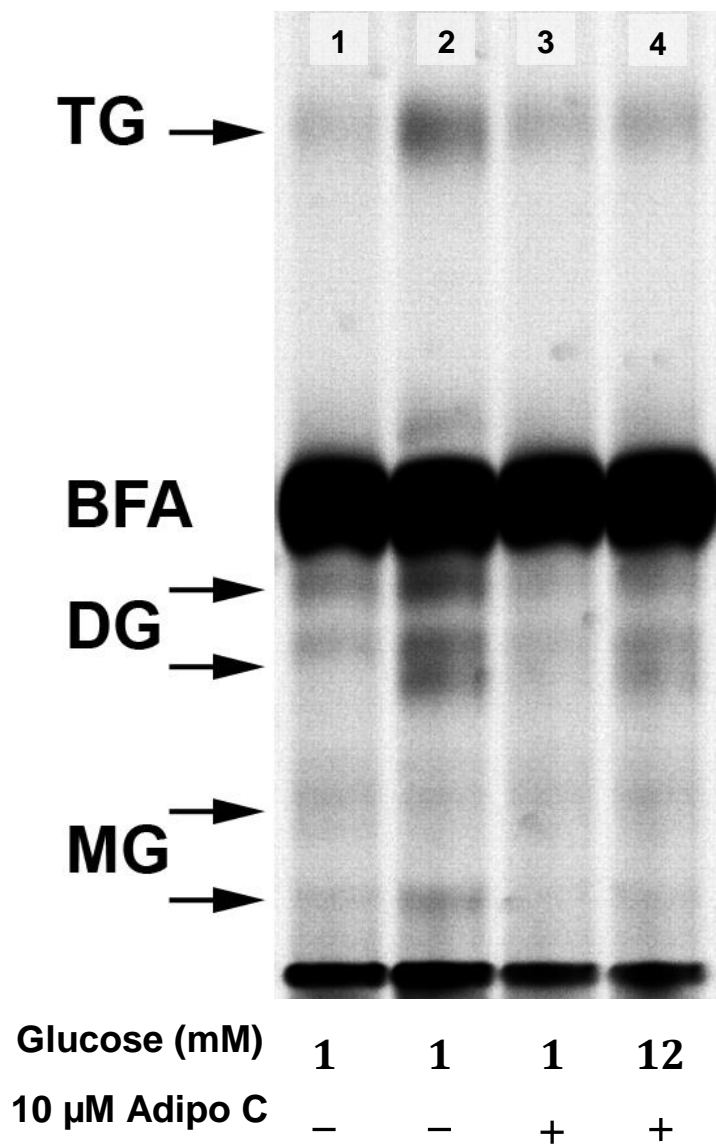


Figure 2. Acute exposure to Adipo C reduces glucose induced lipid incorporation. Thin layer chromatogram (TLC) of lipid extracts with BODIPY fatty acid from INS-1 cells incubated in 1 or 12 mM glucose in the presence or absence 10 μ M Adipo for 2 hrs. Triglyceride (TG), diglyceride (DG), and monoglyceride (MG) are indicated by arrows. Control was 0 μ M Adipo C with 0.1 % DMSO.

Adipo C reduces intracellular lipid droplets

Nile red was used to stain intracellular TG lipid droplets in INS-1 cells cultured in 4 mM glucose and cells switched from 4 mM to 11 mM glucose for 72 hr with and without 10 μ M Adipo C (Figure 3). Cells chronically cultured in 4 mM glucose (panel A) exhibited less lipid droplets (represented by bright dots) compared to cells switched to 11 mM glucose for 72 hrs (panel C), consistent with previous data from our lab (Erion) (42). Cells chronically cultured in 4 mM glucose media with 10 μ M Adipo C exhibited no apparent difference in lipid droplet level compared to the control (compare panel B to panel A). Cells switched to 11 mM glucose media with 10 μ M Adipo C showed reduced level of lipid droplets compared to 11 mM glucose control condition (compare panel D to panel C). This result seems to be due to a reduction in both the number and size of lipid droplets.

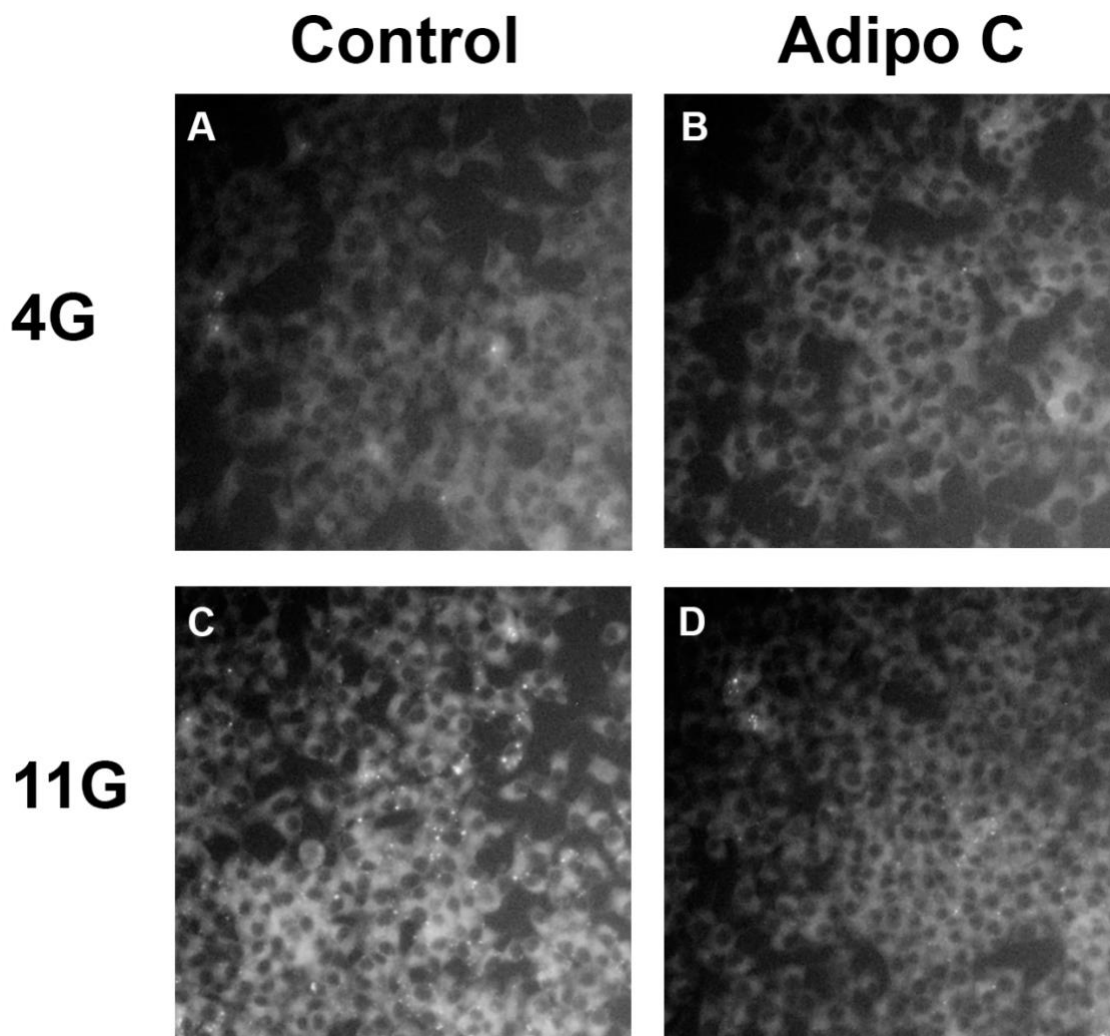


Figure 3. Chronic exposure to adipo C reduces lipid droplets in INS-1 cells. INS-1 cells were cultured in 4G with (B) and without (A) Adipo C, or in 11G with (D) and without (C) Adipo C. for 72 hrs. Lipid droplet were assessed with the dye Nile red and representative pictures were taken at 20 \times magnification.

Adipo C exposure increases doubling time of INS-1 cells in culture

INS-1 cells were cultured starting at a know cell density in 96-well plates and the number of cells per well were counted after 4-6 days. Doubling time of INS-1 cells chronically cultured in 11mM glucose RPMI (>2weeks) with or without exposure to

increasing concentration of Adipo C compound for 72 hrs were calculated. Cells chronically cultured in 11 mM glucose (blue bar) replicated at a rate of 34 hrs/ doubling time. Adipo C exposure resulted in a significant decrease in the rate of growth of INS-1 cells increasing the doubling time of cells cultured in 11 mM glucose with 10 μ M (green bar) and 25 μ M Adipo C (red bar) compared to the 11 mM glucose control. The doubling time increased from 34 hrs to 40 hrs and 47 hrs for 10 μ M and 25 μ M Adipo C treated cells, respectively. No significant difference in doubling time was observed between 11G cells cultured with 10 μ M and 25 μ M Adipo C.

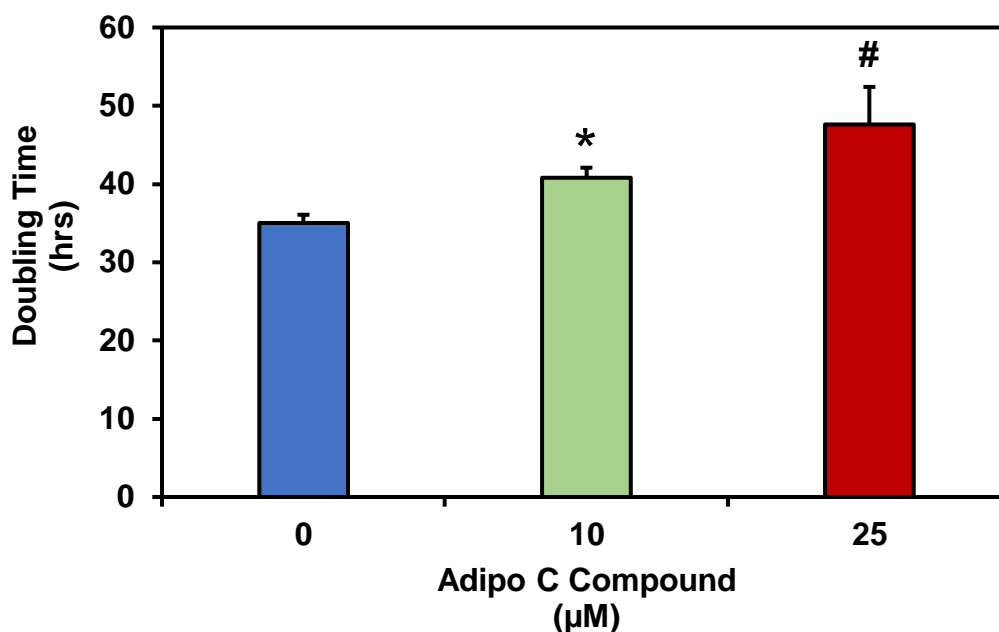


Figure 4. Chronic exposure to Adipo C increases doubling time for INS-1 cells. INS-1 cells were cultured in 0 (blue bar), 10 (green bar) or 25 (red bar) μ M Adipo C for 72 hrs. Doubling time was calculated. Doubling time was measured from 5 independent experiments. Error bars represent \pm SE. (*, $p < 0.01$ compared to control group; #, $p < 0.05$ compared to control group). Control was 0 μ M Adipo C with 0.1 % DMSO.

The effect of Adipo C on glucose induced insulin release

To characterize the effect of Adipo C on glucose induced insulin release, insulin secretion was measured from INS-1 cells cultured in 11 mM glucose RPMI media with and without 72 hrs exposure to increasing concentrations of Adipo C compound (Figure 5). Cells cultured without Adipo C (blue bars) exhibited a dose-dependent increase in GSIS from 1 to 12 mM glucose. Both 3 and 12 mM glucose significantly increased insulin secretion compared to basal (1 mM) glucose. The increase at 3 mM glucose was consistent with Erion et al. demonstrating that INS-1 cells cultured in 11 mM glucose exhibit a left-shift in the glucose dose-response for insulin secretion (42). When cells were exposed to 10 (green bars) or 25 (red bars) μ M Adipo C, basal insulin secretion was increased although this was not statistically significant. In contrast to the control condition, Adipo C treated cells showed no increase in insulin secretion when glucose was increased from 1 to 3 mM glucose. This suggests that Adipo C can reverse the left-shift in insulin secretion from cells cultured in 11 mM glucose. Adipo C increased 12 mM GSIS and this was significant at 25 μ M concentration (red bars).

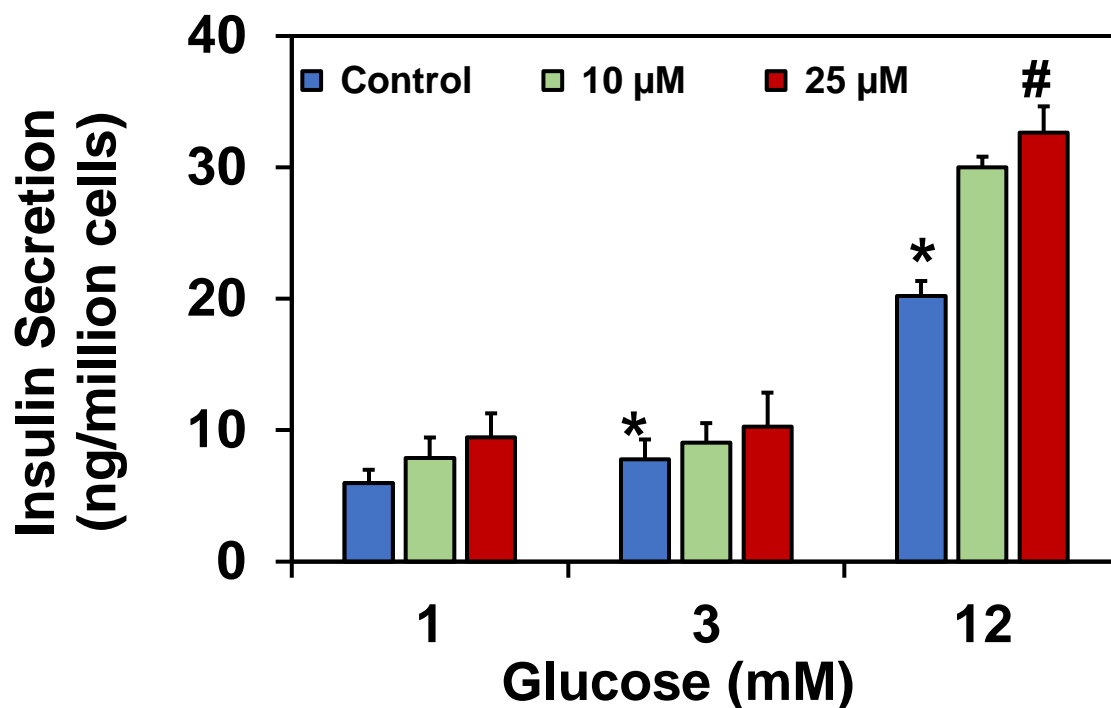


Figure 5. Chronic exposure to Adipo C increases GSIS from INS-1 cells. INS-1 cells were cultured in 0 (blue bars), 10 (green bars) or 25 (red bars) μ M Adipo C for 72 hrs. Insulin secretion was measured at 1, 3 and 12 mM glucose for 2 hours. Insulin secretion was measured from 5 independent experiments. Error bars represent \pm SE. (*, $p < 0.05$ compared to control group at 1 mM glucose; #, $p < 0.05$ compared to control at 12 mM glucose). Control was 0 μ M Adipo C with 0.1 % DMSO.

The changes in basal insulin secretion in cells treated with 10 μ M and 25 μ M Adipo C prompted us to study whether this increase was due to an increase in the level of intracellular Ca^{2+} . Insulin secretion was measured from INS-1 cells cultured in 11 mM glucose RPMI media with and without 72 hr exposure to increasing concentrations of Adipo C compound (Figure 6). INS-1 cells cultured without Adipo C compound had a significant increase in insulin secretion at 3 mM glucose condition compared to 1 mM glucose (Figure 6 blue bars). This increase of insulin secretion is characterized as an

important sign of glucolipotoxicity according to Erion et al (42). The increase was alleviated in INS-1 cells cultured in 11 mM glucose RPMI with 10 μ M (Figure 6 green bars) and 25 μ M (Figure 6 red bars) Adipo C compound in a dose dependent manner. INS-1 cells cultured without Adipo C compound had a significant decrease in insulin secretion in 3mM glucose with diazoxide (DZ) ($K^{+ ATP}$ channel activator) compare to that of 3 mM glucose. Diazoxide showed less or no inhibition in 10 μ M (green bars) and 25 μ M (red bars) Adipo C concentration, which indicates that the insulin secretion of INS-1 cells cultured with Adipo C at 1 and 3 mM glucose is not due to increased Ca^{2+} influx; And the increased insulin secretion from 1 to 3 mM glucose observed in control cells is due to increased Ca^{2+} influx.

Similar trend was seen in INS-1 cells cultured in 4 mM glucose, which is considered a physiological concentration and has better resemblance to islets (39). In 4G cells, insulin secretion at dose-responsive GSIS curve did not show difference between cells cultured with (Figure 7, panel A-green shadowed) or without Adipo C (Figure 7, panel A blue shadowed). In addition, when glucose increased from 1 to 3 mM, insulin secretion did not increase in both 4G cells cultured with (Figure 7, panel C-green shadowed) or without Adipo C (Figure 7, panel C-blue shadowed). After switch 4G cells to 11G RPMI media for 72 hrs without Adipo C , GSIS was blunted (Figure 7- panel B blue bars) whereas cells cultured with Adipo C (Figure 7- panel B green bars) showed restored GSIS. In contrast with chronic 4G cells, 4G switched to 11G without Adipo C (Figure 7- panel B blue bars) has an increase in secretion when glucose level was elevated from 1 to 3 mM. When 4G cells were exposed to with 10 μ M Adipo C for 72 hrs, the increase at 3 mM glucose

compare to 1 mM glucose was blunted. Taken together, data on 4G cells transferred to 11 mM glucose RPMI for 72 hrs with or without Adipoc is in great consistency with Erion et al. (42) and chronic 11G cells (figure 5, 6), which showed that 11G exhibited a left-shift in the glucose dose-response for insulin secretion and this secretion is, at least partially, rescued by Adipo C compound.

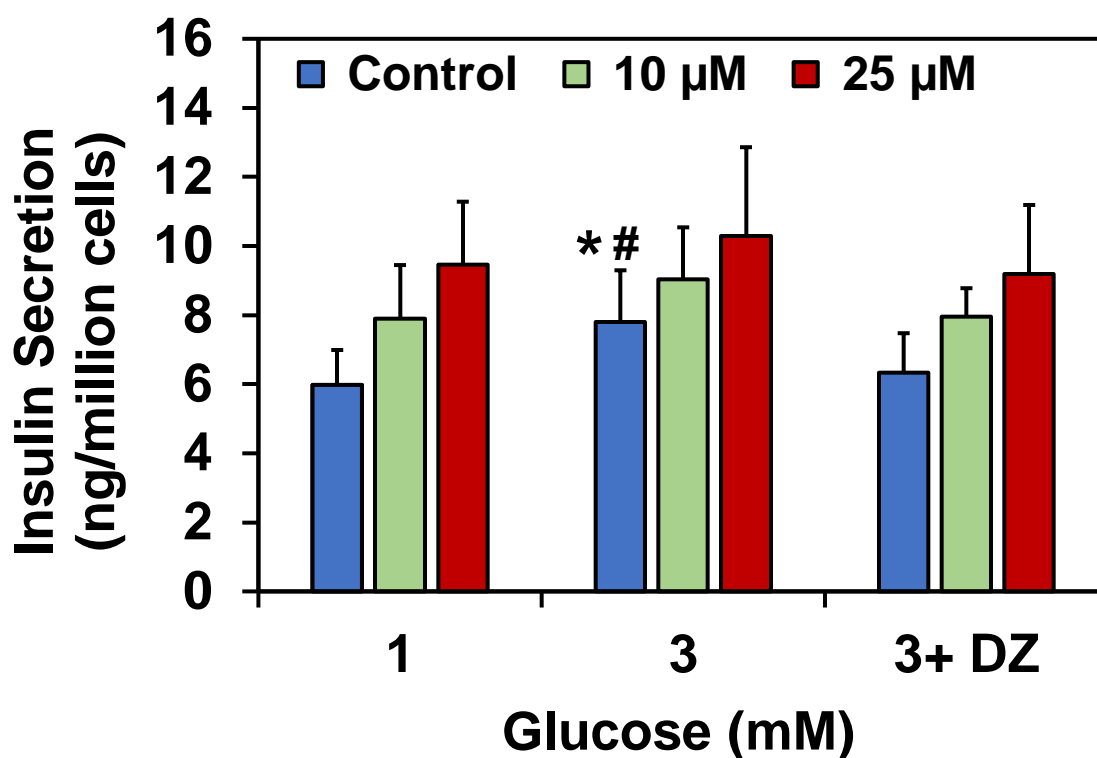


Figure 6. Chronic exposure to adipo C alleviate increased basal insulin secretion in a dose dependent manner. INS-1 cells were cultured in 0 (blue bars), 10 (green bars) or 25 (red bars) μM Adipo C for 72 hrs. Insulin secretion was measured at 1, 3 mM glucose and 3mM glucose with Diazoxide (DZ) for 2 hours. Insulin secretion was measured from 5 independent experiments. Error bars represent \pm SE. (*, $p < 0.05$ compare to control group at 1 mM glucose; #, $p < 0.05$ compare to control at 12 mM glucose). Control was 0 μM Adipo C with 0.1 % DMSO.

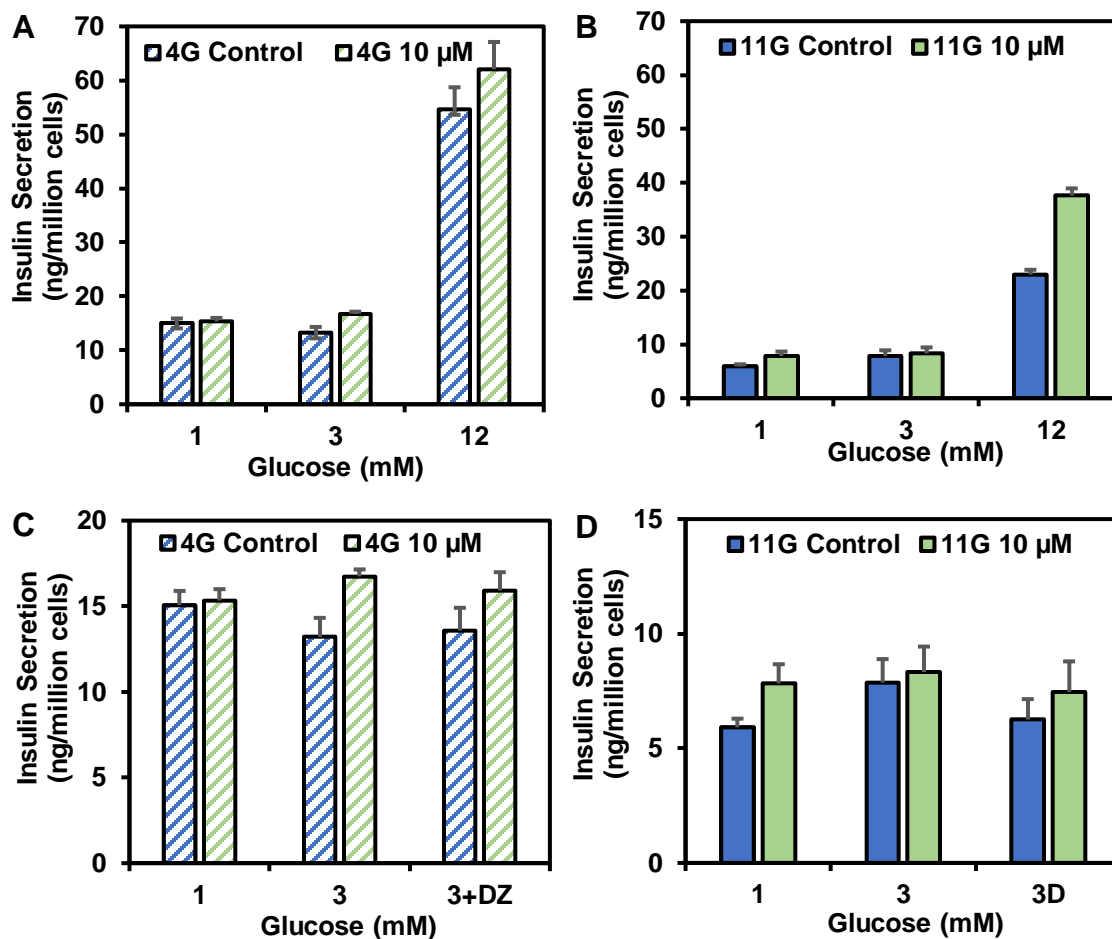


Figure 7 Chronic exposure to Adipo C rescues reduced GSIS and alleviates increased basal insulin secretion in 11G cells. INS-1 cells were cultured in 4 mM glucose RPMI and then transferred to 0 (blue bars), 10 (green bars) μ M Adipo C for 72 hrs in 4 mM or 11 mM RPMI. Insulin secretion was measured at 1, 3, 12 mM glucose and 3 mM glucose with Diazoxide for 2 hours. Insulin secretion was measured from 1 independent experiment. Error bars represent \pm SE. Control was 0 μ M Adipo C with 0.1 % DMSO.

Adipo C increases insulin Content

To determine whether the increase of insulin release at high glucose is due to increased insulin content, we measured total insulin from INS-1 cells cultured in 11 mM glucose RPMI media with and without 72 hr exposure to increasing concentrations of Adipo C compound (Figure 8). INS-1 cells cultured with 10 μ M Adipo C (green bar) had a 25% increase in insulin content compared to the control group (blue bar). Insulin Content in INS-1 cells cultured with 25 μ M Adipo C (red bar) increased 84% compared to the control group.

Insulin secretion was then calculated as a percentage of insulin content from INS-1 cells (Figure 9). GSIS as a percentage of the total insulin content was similar among the three conditions (control, 10 μ M Adipo C, 25 μ M Adipo C). A similar trend was observed across insulin secretion at 1 mM glucose and 3 mM glucose. This suggests the increased insulin secretion at 3 mM and 12 mM glucose in Adipo C treated groups observed in Figure 6 largely results from increased insulin content.

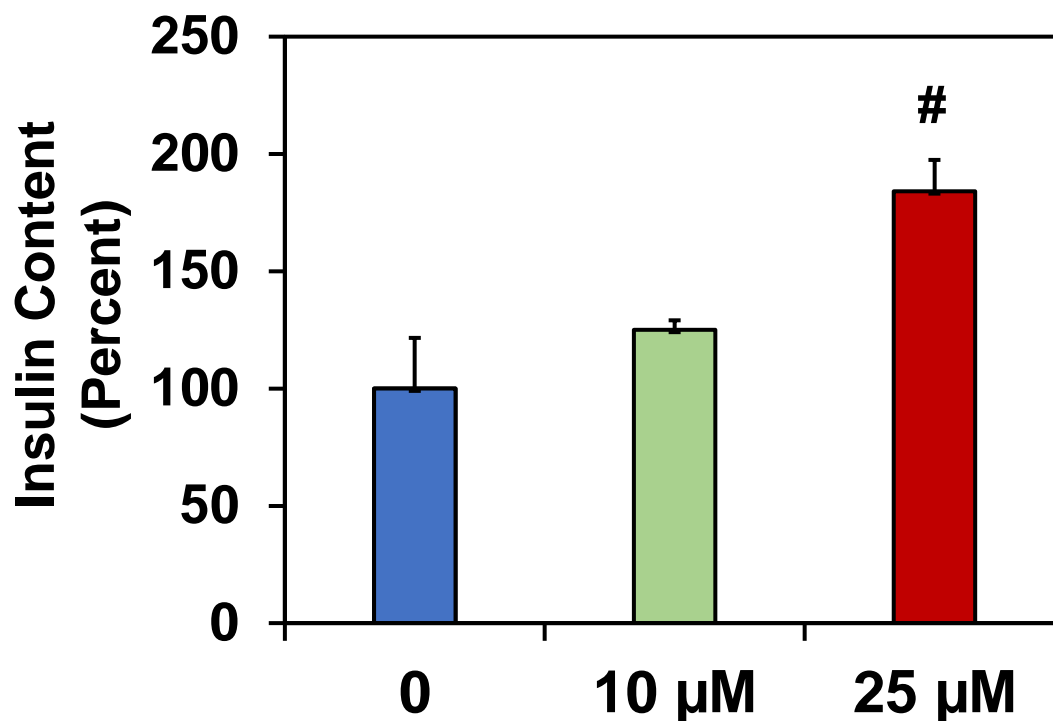


Figure 8. Chronic exposure to Adipo C increases insulin content in INS-1 cells. INS-1 cells were cultured in 0 (blue bars), 10 (green bars) or 25 (red bars) μM Adipo C for 72 hrs. Insulin content was calculated as a percent of the control without Adipo C, which was set at 100 %. Insulin content of INS-1 cells without Adipo C was used as 100 %. Insulin content was measured from 5 independent experiments. Error bars represent \pm SE. (*, $p < 0.05$ compare to control group at 1 mM glucose; #, $p < 0.05$ compare to control at 12 mM glucose). Control was 0 μM Adipo C with 0.1 % DMSO.

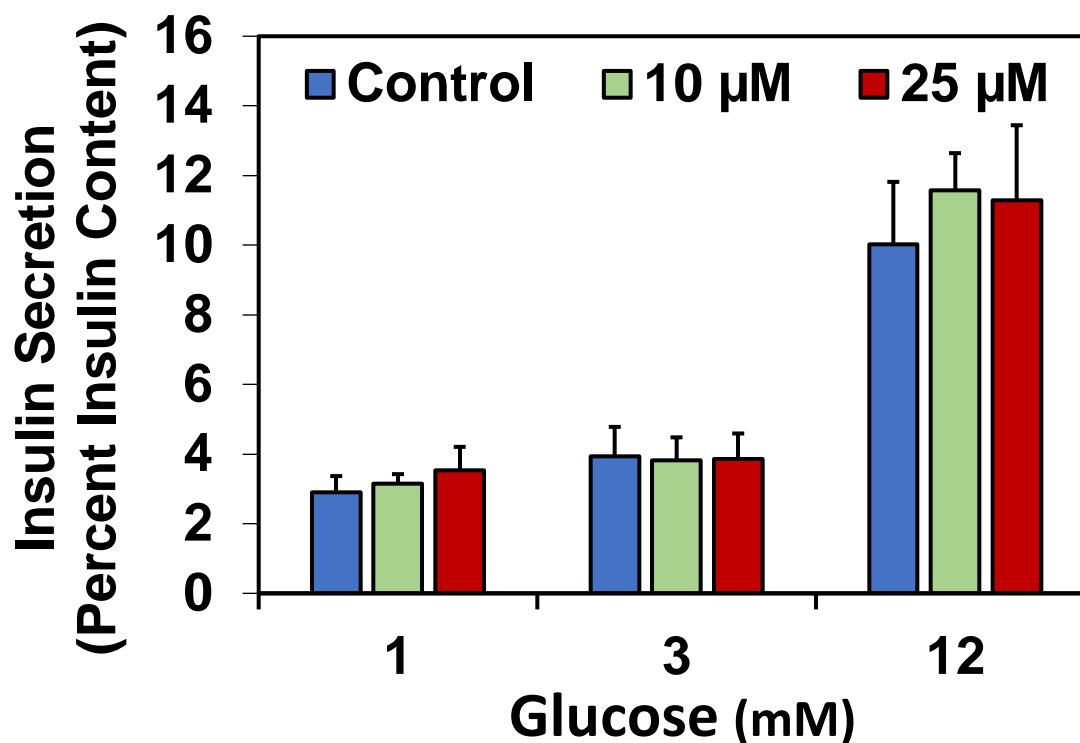


Figure 9. Chronic exposure to Adipo C increases GSIS through increased insulin content. INS-1 cells were cultured in 0 (blue bars), 10 (green bars) or 25 (red bars) μM Adipo C for 72 hrs. Insulin secretion was measured at 1, 3 and 12mM glucose (Figure 6) and then calculated as a percent of total insulin content. Insulin secretion and insulin content was measured from 5 independent experiments. Error bars represent \pm SE. Control was 0 μM Adipo C with 0.1 % DMSO.

Intracellular Ca²⁺ measurement

Figure 7 shows that cells switched from 4 to 11 mM glucose exhibited a left-shift in glucose-induced secretion, which was abolished by diazoxide (compare blue bars in panel C and D). Since it was observed that Adipo C prevented this left-shift in secretion we were interested in examining intracellular Ca²⁺ activity in response to the same glucose concentrations (Figure 10). The extracellular glucose was raised from 1 mM glucose (0-15 min) to 3 mM glucose (15-30 min) and then to 12 mM glucose (30 min-end of trace) in fura 2 loaded cells. Cells cultured in 4 mM glucose (Figure 10, panel A) and cells switched from 4 to 11 mM glucose in the absence (Figure 10, panel B) or presence of 10 μ M (Figure 10, panel C) and 25 μ M (Figure 10, panel D) Adipo C for 72 hrs were imaged.

Cells in 11 mM glucose in the absence of Adipo C (Figure 10, panel B) exhibited apparent increase in Ca²⁺ activity when glucose level was raised from 1 to 3 mM but failed to further increase the level of Ca²⁺ when it was switched to 12 mM glucose. Whereas cells cultured in 4 mM glucose barely responded to the same low glucose increase (1 to 3 mM) but exhibited high level of Ca²⁺ activity in response to stimulatory glucose level (12 mM glucose) (Figure 10, compare panel A to B). Cells grown in 11G and 10 μ M Adipo C (Figure 10, panel C) had no obvious increase in Ca²⁺ activity level when glucose was increased from 1 to 3 mM resembling 4 mM glucose cells, but showed a lag in increasing Ca²⁺ activity at 12 mM glucose level compare to 4G cells (Figure 10, compare panel C to A). 11 mM glucose cells cultured with 25 μ M Adipo C (Figure 10, panel D) presented a better resemblance to the 4 mM glucose control group both in lack of basal Ca²⁺ activity (from 1 to 3 mM glucose) and an active stimulatory response at 12 mM glucose that

resulted in a more rapid rise in intracellular Ca^{2+} (Figure 10, compare panel D to A). Similarly, 11G cells culture in the presence of 25 μM Adipo C (Figure 10, panel D) exhibited damped oscillation at 1 and 3 mM glucose but a quicker and more active response when stimulatory glucose was added compare to 11G cultured in the absence of Adipo C (Figure 10, compare panel D to B).

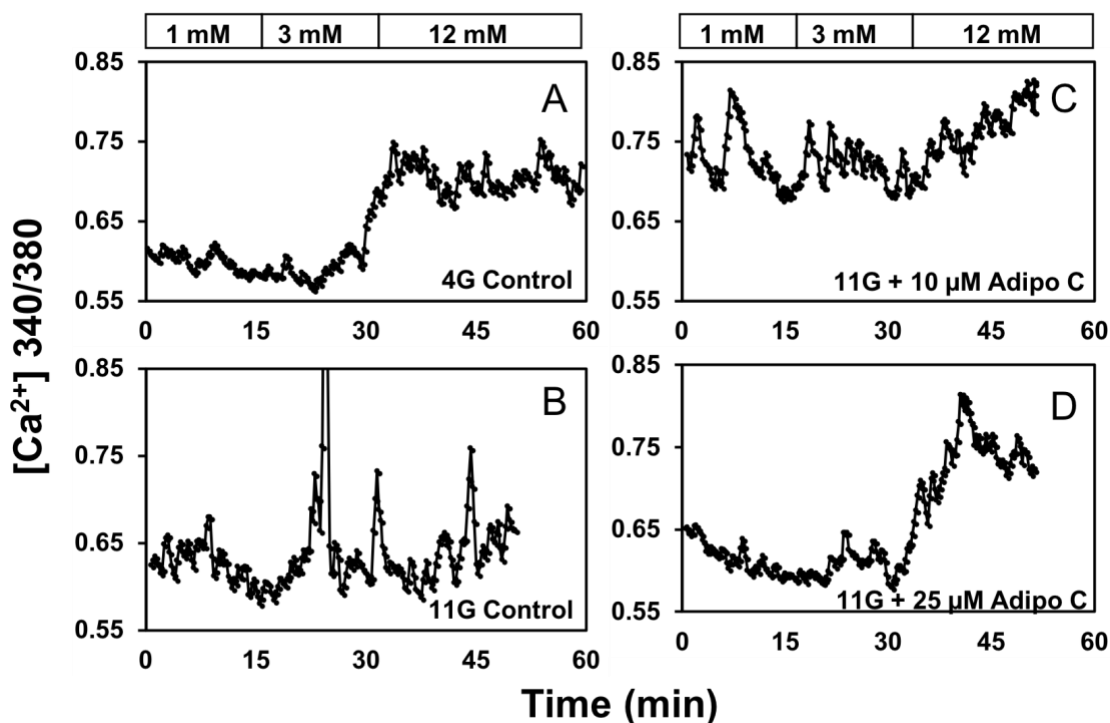


Figure 10. Chronic exposure to Adipo C changes the oscillating pattern of intercellular Ca^{2+} . 4G cells were transitioned to 4 mM glucose media (panel A), 11 mM glucose in the absence (panel B) or presence of 10 μM (panel C), 25 μM (panel D) Adipo C for 72 hrs. Intercellular Ca^{2+} level was measured as a ratio of fluorescence at 340/380 dual excitation. Measurement was taken every 15s for at least 45 mins, consisted of 31 cells per trace, including 1 mM glucose (0-15 mins), 3 mM glucose (15-30 mins), 12 mM glucose (30 mins - end of trace). Control was 0 μM Adipo C with 0.1 % DMSO.

Insulin Oscillation

In order to determine whether Adipo C affected the oscillatory pattern of insulin secretion we performed column perfusion experiments. INS-1 cell spheres were incubated with (red line) or without (blue line) 25 μ M Adipo C for 72 hrs, loaded onto columns of cytodex beads and perfused with varying glucose concentrations while collecting 15 sec fractions for insulin analysis. Spheres were perfused with 1 mM glucose (1-20 mins), 3 mM glucose (20-40 mins), 12 mM glucose (40-60 mins) and 12 mM glucose with 30 mM KCl (60-72 mins). Both Control and Adipo C treated cells exhibited a regular oscillatory pattern. Oscillations from control cells were of higher amplitude throughout the time course of secretion. The pattern of oscillations was altered in the control cells after increasing glucose from 1 to 3 mM. This could be due a left-shifted glucose response in these cells. This altered pattern was less obvious in cells treated with Adipo C. Cells cultured with Adipo C (red line) for 72 hrs exhibited a robust increase in insulin secretion to 0.4 ng/ml with stimulatory glucose (12 mM) glucose and continued to oscillate at that level. The increase in secretion observed in control cells (blue line) was very much blunted. The difference in the oscillatory amplitude was marked when cells were depolarized with KCl, with control cells exhibiting larger amplitude oscillations compared to cells treated with Adipo C. Taken together, cells cultured with Adipo C exhibited a higher insulin secretion level compare to cells cultured in control condition, with similar period but reduced amplitude oscillations.

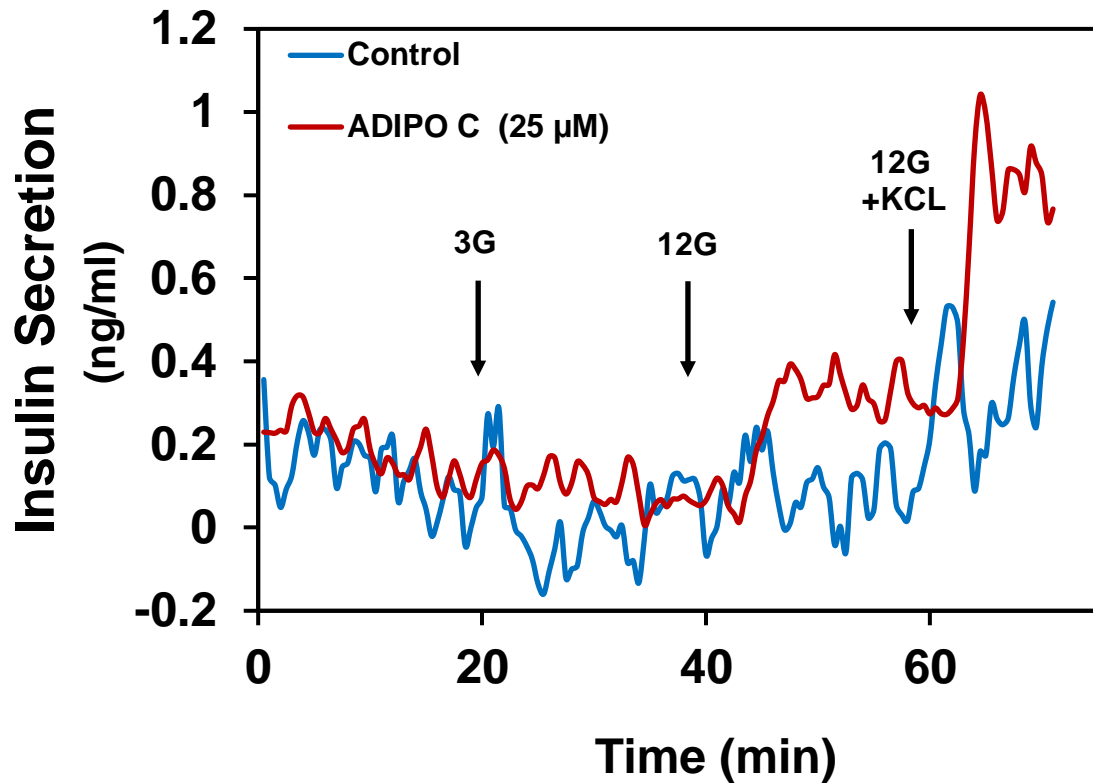


Figure 11 Chronic exposure to Adipo C does not change oscillatory pattern of insulin secretion but decrease the amplitude. INS-1 cells formed colonies were cultured in 11 mM glucose media with (red line) or without (blue line) 25 μM Adipo C for 72 hrs. Insulin secretion was measured over a time lapse of 72 mins with glucose increasing from 1 mM (20mins), 3mM (20mins) to 12 mM glucose (20mins) and 12 mM glucose with 2 mM KCL (12 mins). Control was 0 μM Adipo C with 0.1 % DMSO. Make calculations of the level and range.

DISCUSSION

In this thesis we have explored the effects of a compound that is thought to inhibit ACSL5 on lipid accumulation and insulin secretion from clonal pancreatic β -cells. We have named this putative inhibitor compound Adipo C. Adipo C exhibited the ability to acutely inhibit fatty acid incorporation into INS-1 cells while reducing intracellular lipid stores in these cells after 72 hrs incubation. Reducing lipid in INS-1 cells using this inhibitor mimicked many of the same changes that we have observed when intracellular lipid was reduced by lowering the glucose concentration in culture (42). These changes include a reduced growth rate, increased insulin content, a more rapid and robust intracellular Ca^{2+} response to high glucose, a right-shift in the glucose dependent insulin secretion and increased GSIS. Some changes that were not seen before include decreased amplitude oscillations in both intracellular Ca^{2+} and insulin secretion.

The ability of Adipo C to inhibit ACSL activity has been suggested by the observation that BFA incorporation into neutral lipids of INS-1 cells was dramatically reduced in Adipo C treated cells compared to control. The decreased incorporation was observed for MG, DG and TG and all may play a role in basal insulin hypersecretion and/or GSIS. DG has been shown to stimulate PKC isozymes that influence exocytosis (70) and bind Munc13-1 to stimulate insulin exocytosis (19). TG may play a role by supplying FA as a product of lipolysis, a process that has been shown to be critical for maintenance of normal GSIS (71).

The required specific assay to determine that inhibition of ACS activity is the cause of the reduced incorporation has not been performed at the time of writing this thesis. We still cannot rule out inhibition of acyl transferase activity, which could yield a similar outcome.

The acute effect of Adipo C to reduce lipid incorporation evidenced by TLC, can lead to cellular lipid depletion illustrated by the long-term loss of intracellular lipid droplets. Lipid droplets are a marker of excess cellular lipid and an indicator of potential glucolipototoxicity (42). The ability of Adipo C to reduce the lipid droplets gives it potential to protect cells from the glucolipototoxic effect.

While short-term exposure of FA to islets or β -cells potentiates GSIS, chronically increased lipid level in INS-1 cells causes a left shift in the glucose dose response of secretion characterized by elevated basal insulin secretion and blunted GSIS (42). Our results showed in cells chronically cultured in 11 mM glucose or switched from 4 to 11 mM glucose for 72 hr (exploratory data), insulin secretion was increased when glucose was raised from 1 to 3 mM. This represents a left shift in secretion when compared to the lack of response in cells cultured in 4 mM glucose. Adipo C prevented this left shift in secretion and increased GSIS in cells cultured in high glucose. Taken together, Adipo C compound showed the ability to reverse or alleviate the effects of GLT and prevent basal insulin hypersecretion that may contribute to hyperinsulinemia and the progression of insulin resistance.

Reduced lipid droplet level clearly affected INS-1 cells in multiple ways. The growth rate of INS-1 cells is slowed down and both GSIS and insulin content increased.

Notably, the altered insulin content is of great importance when interpreting insulin secretion and functional capacity of β -cells under GLT as highlighted by Erion et al (42). Increased insulin secretion from Adipo C treated cells came to the same level as controls after normalizing secretion to insulin content. This suggested to us that the effect of Adipo C on insulin secretion may be due to increased insulin content. It has been shown that some lipid moieties such as ceramide induced by palmitate inhibit insulin transcription thereby reducing insulin biosynthesis (31)(30). Therefore, the increased insulin content in cells exposed to Adipo C is potentially associated to reduced fatty acid incorporation into lipids. Further studies on the extent of inhibition on ceramide synthesis caused by Adipo C are needed to verify this hypothesis.

It has been previously shown that cells cultured in low glucose respond with a rapid and robust intracellular Ca^{2+} response compared to cells exposed to 11 mM glucose. The increased Ca^{2+} influx in cells cultured at 4 mM glucose correlates well with the increased secretion from these same cells. Our Ca^{2+} traces showed that cells cultured in 4 mM glucose have minimal Ca^{2+} activity at 1 and 3 mM glucose and rapidly and robustly increased at 12 mM glucose consistent with the glucose induced secretion (Fig. 6 panel A blue bar). In comparison, cells cultured in 11 mM glucose media alone exhibited the significant increase in secretion from 1 to 3 mM glucose, whereas when glucose was raised to 12 mM glucose they exhibited attenuated response to Ca^{2+} and reduced GSIS, which is also consistent with its secretion (Fig. 6 panel C blue bar). Comparison of cells cultured in 4 mM glucose and 11 mM glucose illustrates the effect of glucolipotoxicity on β -cells. Our results showed striking resemblance between cells cultured in 11 mM glucose with 25 μM Adipo C

compound and cells cultured in 4 mM glucose. This serves as strong support for the ability of Adipo C compound to protect β -cells from basal hyper insulin secretion that may participate in the progression of insulin resistance.

There are possible pitfalls of average Ca^{2+} activity measurement: 1) instead of looking at individual cells, the average is used to represent the level of Ca^{2+} activity in 31 separate cells; 2) oscillations would be artificially reduced or diminished during the calculations; and 3) the average Ca^{2+} could mask oscillations due to lack of synchrony among cells.

The oscillations in insulin release are of great importance as the oscillatory pattern is impaired in T2D patients (22). The kinetics of insulin secretion revealed similar period but altered pattern of oscillation between cells cultured with or without Adipo C. The frequency of insulin oscillations seems unaffected, indicating glucose metabolism coupled to secretion is intact (20). The amplitude at which they oscillate was seemingly reduced by exposure to Adipo C. This may be the result of lack of synchrony between spheres in columns, which could mask actual oscillation.

So far the data we have collected demonstrated that the putative inhibition effect of ACSL5 may increase GSIS and reverse the left shift of insulin secretion caused by chronic exposure to excess nutrients (42). Other studies have shown using triacsin C, a long-chain acyl-CoA synthetase inhibitor, that reducing LC-CoA does not inhibit insulin secretion despite changes in fatty acid oxidation and palmitate incorporation into lipids (51). The discrepancy between their data and our data can be explained by the difference in specificity of these two inhibitors. Triacsin C is capable of inhibiting ACSL1 and ACSL4

but not ACSL5 (73), but Adipo C is considered as a putative specific ACSL5 inhibitor. Different ACSLs vary in their functions and locations inside the cells and it is unlikely that inhibiting different ACSLs will produce the same result.

Adipo C compound showed protective effect of decreasing intrinsic lipid accumulation, preserving insulin content and reversing left shift of glucose induced insulin secretion from clonal pancreatic β -cells grown in glucolipotoxic environment and no adverse effect on clonal pancreatic β -cells grown in physiological environment. The desirable effect of Adipo C on β -cells warrants further research on Adipo C compound to explore its potential as a possible therapeutic agent for T2D patients

LIST OF JOURNAL ABBREVIATIONS

Am J Physiol Endocrinol Metab	American Journal of Physiology. Endocrinology and Metabolism
Annu Rev Med	Annual Review of Medicine
Annu Rev Nutr	Annual Review of Nutrition
Arch Biochem Biophys	Archives of Biochemistry and Biophysics
Biochem J	Biochemical Journal
Biochim Biophys Acta	Biochimica et Biophysica Acta
Cell Metab	Cell Metabolism
Curr Opin Clin Nutr Metab Care	Current Opinion in Clinical Nutrition and Metabolic Care
Diabet Med	Diabetic Medicine
Diabetes Metab Res Rev	Diabetes/Metabolism Research and Reviews
Diabetes Res Clin Pract	Diabetes Research and Clinical Practice
Epidemiol Rev	Epidemiologic Reviews
J Biochem (Tokyo)	Journal of Biochemistry
J Biol Chem	Journal of Biological Chemistry
J Clin Endocrinol Metab	Journal of Clinical Endocrinology and Metabolism
J Clin Invest	Journal of Clinical Investigation
J Intern Med Suppl	Journal of Internal Medicine. Supplement
Lancet Glob Health	The Lancet. Global Health
Mol Cell Biochem	Molecular and Cellular Biochemistry
Mol Cell Endocrinol	Molecular and Cellular Endocrinology
Mol Metab	Molecular Metabolism

N Engl J Med

New England Journal of Medicine

Nat Genet

Nature Genetics

Physiol Rev

Physiological Reviews

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CURRICULUM VITAE

