國立臺灣大學醫學院生物化學暨分子生物學研究所

碩士論文

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Rutin 與 EGCG 作用於大鼠胰臟 β 細胞之抗糖毒性機制探討

Protection of Rat Pancreatic β cells Against Glucotoxicity by Rutin and EGCG

蔡佩珊

Pei-Shan Cai

指導教授：林仁混 博士

Advisor: Jen-Kun Lin, Ph.D.

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中文摘要

第二型糖尿病，此後天慢性疾病對於現今人類有著重大的威脅，目前以周邊組織產生胰島素抗性與胰臟胰島β細胞功能喪失為主要探討之對象。在長時間因飲食等因素導致肥胖產生下，當體內為因應高代謝負荷量時，胰島β細胞會大量釋出胰島素促使糖類代謝，過去研究發現在肥胖個體上胰島β細胞因應體內代謝需求，產生代償性β細胞增生之現象，如此達到應對體內代謝之需求。但在長期負荷環境下可導致胰島β細胞失去對糖份感受性，最終可促使β細胞走向細胞凋亡一途，當胰島β細胞失去代償作用，同時合併周邊組織胰島素抗性之情況下，往往成為糖尿病致死之因素。過往研究中已顯示茶中富含之EGCG對於抗癌與身體能量之代謝有著顯著之功效。此外，已有研究顯示，蕎麥濃縮萃取物可有效降低於糖尿病模式動物之血糖濃度。因而針對天然物質中茶多酚物EGCG與蕎麥富含之類黃酮Rutin，對於胰島β細胞於高糖濃度的環境下，是否能提供實質上保護作用與其分子機制為何，進行相關研究與探討。

於本實驗研究顯示，EGCG與Rutin皆可以有效增加β細胞對於高糖環境培育下之胰島素釋放，並對長時間培育於高糖環境下之β細胞維持對糖份之感受性，並有效減緩β細胞長期培育於高糖環境下所導致之細胞衰亡之發生，有助於細胞生長與存活。進一步探討其分子機制，Rutin與EGCG可有效促進其PDX-1轉錄因子進入細胞核，並促進其活化。PDX-1為對於胰島發育與β細胞維持正常功能之重要因子，有效活化PDX-1可幫助β細胞生長、胰島素生成與第二型葡萄糖運輸蛋白(Glucose transporter 2)等蛋白質表現，同時發現EGCG於長時間的作用下，更可有效增加PDX-1蛋白質表現量。胰島素接受器受質IRS-2在過去研究顯示，對於β細胞生長、維持糖份感受性和胰島素生成等相關生理功能皆扮演重要角色，而β細胞長期處於高糖環境下會降低IRS-2表現而影響細胞正常功能，在同時給予細胞Rutin與合併於高糖的環境下，發現對於胰島素接受器受質(IRS-2)蛋白質表

現量有顯著增加的效果，對於其活性的增加也藉由Rutin與EGCG之給予有著顯著的效果，並有效影響至下游訊號，包括Akt與FoxO1活性之表現。過去研究顯示β細胞在長期高糖環境下會促進細胞內脂肪堆積，造成對細胞的損傷與破壞，直接影響其正常功能運作。EGCG及Rutin於高糖之環境下對於β細胞皆能快速促進活化細胞內之AMPK(AMP-activated protein kinase)此激酶活性，並能有效抑制脂肪酸合成酶FAS(Fatty acid synthase)之生成，同時也抑制乙醯輔酶A羧化酶ACC(Acetyl-CoA carboxylase)活性之上升與脂質合成相關之轉錄因子SREBP1的表現，並有效減緩細胞於高糖環境下細胞內脂肪之囤積。Rutin與EGCG有效幫助胰島β細胞於調控醣類與脂質類代謝，此外，也可藉由影響細胞週期相關之蛋白質表現幫助維持細胞活性與增生，如Cyclin D1與p21，同時也作用於BAX與Bcl-2等蛋白質表現，幫助抑制高糖下導致之細胞凋亡現象。

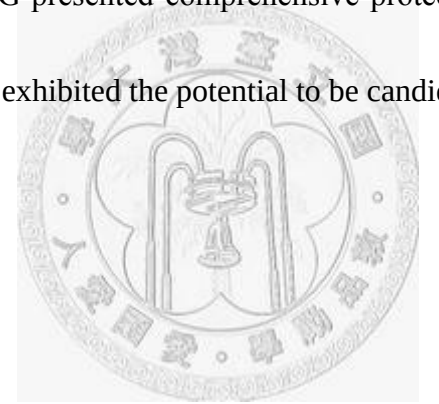
胰島β細胞於長期高糖環境下，促使其逐漸失去正常生理功能與反應，而EGCG與Rutin兩種物質則可幫助β細胞面對於高糖所誘導產生之毒性，延續其細胞活性與存活能力，可望有效延緩糖尿病致病過程之發展。

Abstract

Pancreatic β cell is a fundamental element for the development of diabetes. Chronic hyperglycemia is associated with insulin insufficiency and peripheral insulin resistance, in which β cells have to meet overloaded metabolic demands, but gradually will cause the deteriorating cell function, even leading to irreversible damage, cell death. The decompensation of pancreatic β cell followed by adaptation of increased demand represents the onset of diabetic progression. Therefore, how to maintain the intact cellular function under long term glucose induced toxicity could be strategies for detaining the progression of diabetes.

Rutin and EGCG, natural occurring compounds, have been abundantly found in buckwheat and tea separately, which have been shown the potential of anti-diabetes and anti-obesity in past studies. The actions of Rutin and EGCG on pancreatic β cell are discussed in this study, manifesting the underlying molecular mechanism in regulating the cellular glucose and lipid metabolism. Rutin and EGCG preserved the glucose sensing and glucose-stimulated insulin secretion ability under high glucose incubation. IRS2 signaling was enhanced in the actions of Rutin and EGCG, facilitating the delivery to downstream signals Akt, FoxO1, and PDX-1, which have been implicated as crucial factors in pancreatic β cell growth and function. AMPK is

considered as a fuel sensor that enables to response the cellular energy expenditure, and also exerts numerous regulations in metabolism. AMPK was activated in the treatment and effectively suppressed the cellular lipogenesis *via* inhibition of FAS expression, inactivation of ACC, and manipulation of SREBP1 maturation. Cyclin D1, p21, Bcl-2, and BAX expression levels are also affected in the treatment of Rutin and EGCG, which enhance the cell viability to deal with chronic exposure of elevated glucose. Long term action of glucose caused multiple abnormalities in metabolism, however, Rutin and EGCG presented comprehensive protection on pancreatic β cell against glucotoxicity, and exhibited the potential to be candidates for anti-diabetes.



Abbreviations

ACC, acetyl-CoA carboxylase

AMPK, AMP-activated protein kinase

CaMMK, calmodulin-dependent protein kinase kinase

Cdk, Cyclin-dependent kinase

EGCG, (-)-epigallocatechin-3-gallate

FAS, fatty acid synthase

FoxO1, Forkhead-O transcription factor 1, FKHR

GK, glucokinase

GLUT2, glucose transporter 2

GSIS, glucose-stimulated insulin secretion

HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-coenzyme A reductase

IRS2, insulin receptor substrate protein 2

MCD, malonyl-CoA decarboxylase

MODY, maturity-onset diabetes of the young

PDX-1, pancreas-duodenum homeobox-1

PEPCK, phosphoenolpyruvate carboxylkinase

SREBP1, sterol-regulatory-element-binding transcription factor 1

TZD, thiazolidinedione

Introduction

Diabetes mellitus is the most common metabolic disorder that causes about 5% of all death globally each year (1). The etiology of diabetes could be genetics or environmental influences (2), including MODY (maturity-onset diabetes of the young) (3), deregulated glucose sensing or insufficient insulin secretion (pancreatic β cell dysfunction), autoimmune-mediated diabetes (type 1), or insulin resistance in the peripheral tissue. Type 2 diabetes comprises 90% of diabetic individuals and its epidemic expansion associated with the diet behavior and the problem of obesity (4, 5). Two major characteristics of type 2 diabetes are pancreatic β cell dysfunction and peripheral insulin resistance (6), which both could arise from imbalance energy metabolism, however, which one is the cause or the consequence of type 2 diabetes that still needs to be resolved. To discard the controversy, pancreatic β cell indeed play a critical role in the pathogenesis of type 2 diabetes (7-9). The failure of pancreatic β cell after adaptation resulted from high metabolic demand (6), that leads to decompensation and accelerates the progression of diabetes accompanied with the higher mortality (10, 11).

The chronic stress of hyperglycemia forced pancreatic β cell to compensate for the continuously increased metabolic load and eventually resulted in dysregulation of

glucose and lipid homeostasis. Insulin is secreted from the islet cells with glucose stimulation to maintain the normal blood glucose levels; however, β cells contribute to insufficient secretion of insulin to meet the chronic hyperglycemia. Impaired glucose-stimulated insulin secretion (GSIS) is the first event of pancreatic β cell dysfunction and the defect could accompany by deterioration of mitochondrial ATP production (12). Pancreatic β cell act as a glucose sensor and the metabolism-secretion coupling mechanism is triggered by glucose transportation, glycolysis, ATP production, depolarization of mitochondrial membrane, calcium ion influx, and the final step of the exocytosis of insulin. The Elevation of ATP/ADP ratio generated from the glucose flux is a crucial factor to regulate glucose-stimulated insulin secretion (13), thus the blockade of oxidative mitochondrial metabolism would directed to inhibit the insulin secretory machinery.

Glucose or carbohydrate is regard as fuel for life; nevertheless, prolonged exposure of high glucose condition would lead body to a pathological circumstance called glucotoxicity. Impairment of glucose-stimulated insulin secretion (GSIS), gradually diminished the insulin/IRS signaling and cell failure are results from the glucose induced toxicity to pancreatic β cell. The mechanism of glucotoxicity involved in several aspects of energy homeostasis, and one of which is the deterioration of IRS2 signaling. IRS2, insulin receptor substrate protein 2, regulates β

cell growth, development, function and survival. Furthermore, IRS2 acts as a critical role in the maintenance of peripheral insulin sensitivity, and central leptin sensitivity (14, 15). Disruption of IRS2 would cause the spontaneous apoptosis of pancreatic β cell and the mice lacking *Irs2* display similarities to diabetic individuals, whereas the upregulation of IRS2 might prevent the onset of diabetes in old and obese mice and delay islet destruction (16).

Pancreatic β cell initially compensates for the peripheral insulin resistance and hyperglycemia by expanding the cell mass and insulin secretory capacity; however, the chronic stress of increased metabolic demand could compromise IRS2 function or diminish its downstream signal cascade. It is considered that to stimulate the IRS2 signaling might be a potential therapeutic strategy for diabetes (15), and effectively activate its downstream signal could preserve the integrity of pancreatic β cell. IRS2 mediates the signaling through Akt phosphorylation to inactivate FoxO1 (Forkhead-O transcription factor 1, FKHR) *via* Ser phosphorylation, and which increases PDX-1 (pancreas-duodenum homeobox-1) expression (17). The role of Akt in β cell is considered as an essential factor that regulates cell proliferation, survival and insulin secretion(18), moreover negatively regulates the downstream factor FoxO1 (19). FoxO1, a transcription activator of *pdx1* gene, shuttles between the cytoplasm and the nucleus switched by phosphorylation, and phosphorylated FoxO1 leads to

sequestration in cytoplasm and facilitate the PDX-1 expression. Previous studies indicated that FoxO1 as a repressor of PDX-1 expression (20) and constitutive nuclear expression would prevent β cell hyperplasia and accelerates the onset of diabetes, furthermore, it has been suggested that FoxO1 nuclear exclusion could be a initiator of pancreatic β cell proliferation during the compensation to insulin resistance (21).

The insulin is exclusively expressed in pancreatic β cell and plays a critical role of controlling the whole body metabolic harmony. PDX-1 is a homeodomain protein that can bind to the proximal region of the insulin gene promoter, and involved in the pancreas development(22). The mutation of PDX-1 have been shown to the onset of one form of diabetes (MODY4) (23), in contrast to upregulate the PDX-1 expression could restore pancreatic β cell function in *Irs2* knockout mice (24). The glucotoxicity could diminish the binding activity of PDX-1 mediated by the oxidative stress, in the end, to impair the insulin gene expression. Moreover, disorder of lipid metabolism or called lipotoxicity contributed from chronic metabolic stress would also cause pancreatic β cell dysfunction and cell failure (25), and inhibit the PDX-1 nuclear translocation (26).

Obesity is one well-known risk factor for the development of metabolic syndrome. Abnormalities in lipid metabolism have been considered as contributing factors for type 2 diabetes, which could influence the peripheral tissue insulin

sensitivity and pancreatic insulin secretory capacity (27). In addition to disorder of lipid metabolism, previous studies have indicated that hyperglycemia and hyperlipidemia could synergize in causing toxicity in pancreatic β cell called glucolipotoxicity (28). Pancreatic β cell would lose the adaptation ability under chronic excessive glucose or fatty acid level exposure, which alter the expression level of key transcription factors and enzymes related to metabolic network. Multiple metabolic abnormalities is dysregulation of the AMP-activated protein kinase (AMPK) signaling cascade, which is a heterotrimetic protein and involved in glucose and lipid metabolism (29). AMPK acts as a fuel sensor and could be regulated by multiple mechanisms, which could be activated by upstream kinases LKB1(STK11) or CaMMK (calmodulin-dependent protein kinase kinase) *via* Thr172 phosphorylation (30).

Long-term actions of glucose on pancreatic β cell lipid metabolism were possibly mediated by suppressing or decreasing the AMPK activity (27). AMPK regulates the expression or activity of the lipid metabolism related proteins, such as Acetyl-CoA Carboxylase (ACC), 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), malonyl-CoA decarboxylase (MCD), fatty acid synthase (FAS), moreover, AMPK could inhibit the generation of sterol-regulatory-element-binding transcription factor 1 (SREBP1) (31, 32). Furthermore, numerous studies indicated that the

accumulation of lipid droplets could lead to proceed the dysfunction of pancreatic β cell (28, 33-35). The effect of chronically elevated glucose not only interferes with the glucose metabolism, but the lipid metabolism. In other words, disturbance of the metabolic harmony eventually leads to a comprehensive effect.

Chronic exposure to high glucose condition would induce β cells damage by failing to compensation, and disorder of cellular metabolism caused from the ablation of molecular mechanism. In addition to cell dysfunction, cell mass would dramatically reduce after the decompensation and in the end, the onset of diabetic progression. Pancreatic β cell has exclusive role in metabolism, however, diabetes or other metabolic syndromes could cause the deleterious effect resulted from disorder of energy metabolism. How to preserve the β cell function or delay the onset of progressive diabetes might be a good starting point for diabetes therapeutic strategy.

Tea (*Camellia sinensis*), one of the global most popular beverages, was regarded as potential agent with anti-diabetes and anti-obesity activity (36). Previous studies have shown that tea polyphenols could activate AMPK to attenuate hepatic lipid accumulation (37), and the activation of IRS1 in hepatoma cells (38). Except for the effect of tea, buckwheat (*Fagopyrum esculentum*) concentrate has been shown the ability to reduce blood glucose concentration in diabetic model mice (39, 40), thus in this current study, two natural occurring compounds EGCG (Epigallocatechin gallate)

and Rutin are used, which have been abundantly found in green tea and buckwheat separately, to examine the effect in pancreatic β cell. The investigation of the action of EGCG and Rutin in pancreatic β cell, which to reveal the underlying molecular mechanism of the protection from glucotoxicity.

Materials and Methods

Materials

The pure compound (-)-epigallocatechin-3-gallate (EGCG), Rutin, D-glucose, sodium pyruvate, HEPES, and ycciuv3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide were obtained from Sigma-Aldrich (St. Louis, MO, USA). The anti-PDX1, anti-Foxa2, anti-SREBP1, anti-pTyr and anti-LKB1 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The anti-pAkt (Ser473) and anti-Bax were obtained from Cell signaling Technology, Inc. (Beverly, MA, USA). The anti-pAMPK (Thr172), anti-AMPK, anti-IRS2 and anti-p21 were from Upstate Biotechnology (Lake Placid, NY, USA). The anti-FAS and anti-Cyclin D1 were from BD Bioscience (Franklin Lakes, NJ, USA). The anti-pFoxO1 (Ser256) and anti-FoxO1 were obtained from Abcam Inc. (Cambridge, MA, USA). The anti-pACC (Ser79) was purchased from Transduction Laboratory (Lexington, KY, USA).

Cell culture

RIN-m5F rat insulinoma pancreatic β cells were obtained from the NHRI cell bank (National Health Research Institutes, Taiwan) and maintained in RPMI 1640 containing 11 mM glucose supplemented with 10 mM HEPES, 1 mM sodium

pyruvate, 10% (v/v) fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin (HyClone, Logan, UT, USA) in a humidified 5% CO_2 incubator at 37 ℃.

Immunoprecipitation and Western blotting

Cells were incubated in media containing 11 mM glucose followed by 2 h starvation and treated with 33 mM glucose contained media (defined as high glucose stimulation state) with or without EGCG or Rutin for indicated duration of time. Cells were lysed with buffer (10% glycerol, 1% Triton X-100, 0.1% SDS, 10 mM NaF, 50 mM Tris-HCl, pH 8.0, 5 mM EDTA, 150 mM NaCl, 0.5 mM phenylmethysulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin and 0.5 mM dithiothreitol), and the lysates were centrifuged at 12,000 rpm for 30 min, and then to collect the supernatants as whole cell extracts. For western blotting, equal amounts of total cellular protein (20 μg) were resolved by SDS-PAGE transferred onto polyvinylidene difluoride membranes (Millipore), and probed with primary antibody followed by secondary antibody conjugated with horseradish peroxidase. The immunocomplexes were visualized with enhanced chemiluminescence system (Perkin Elmer life sciences, Boston, MA, USA). For immunoprecipitation, equal amount of cell lysates were precipitated with anti-IRS2 antibodies, and then immobilized on protein G-Sepharose

beads followed by gently rocking overnight at 4 ℃. To collect the immunocomplexes by centrifugation at 12,000 rpm for 30 min at 4 ℃, and then washed by ice-cold PBS, incubated at 100℃ for 10 min with 20 μl electrophoresis buffer, and the supernatant was analyzed by western blotting.

ATP detection assay and insulin secretion detection

The cells were incubated in the media contained with maintained glucose condition (11 mM) or high glucose condition (33 mM) for 48 h, and then to stimulate the cells by replacing the media contained with high glucose condition with or without Rutin or EGCG. After 2 h stimulation, the cell media were obatined to quantification by rat insulin ELISA kit (Mercodia Inc., Uppsala, Sweden), and the ATP contents were quantificated by luminescence ATP detection assay system (Perkin Elmer life sciences, Boston, MA, USA).

Immunohistochemistry

Cells were incubated in high glucose condition with or without Rutin or EGCG for 24 h. Cells were washed with PBS, fixed with 10% formalin for 30 min on ice, washed twice with PBS, and blocked with 1% BSA/PBS for 1 h at room temperature, and then incubated with anti-PDX1 antibodies (100X) at room temperature for 1 h.

The secondary antibody conjugated with FITC (200X, Santa Cruz biotechnology) was used for 1 h incubation at room temperature. The nuclei were stained by DAPI for 1 h and all images were acquired from fluorescence microscope (Carl Zeiss Inc., AXIO Image A1).

Cell cycle Analysis and Flow cytometry

Cells were incubated in 25 mM glucose contained media with or without Rutin or EGCG after 2 h starvation and replaced the media every 24 h. For indicated duration of time, cells were trypsinzed and fixed in PBS with 70% ethanol. After fixation and discard of ethanol, Cells were resuspended in PBS, then treated with 2% Triton X-100 and RNase A incubated at 37 ℃ for 30 min and exposed to propidium iodide (PI). Cell cycle analysis was performed by BD FACSCalibur system (BD Biosciences, Mountain View, CA) and the fraction of cell cycle was calculated by Acquisition software of BD CellQuest (BD Biosciences, Mountain View, CA).

Oil Red O staining

To measure the cellular lipid droplet accumulation, cells were incubated in maintained glucose condition or high glucose condition with or without Rutin or EGCG after 2 h starvation. These cells were incubated for 5 days and replaced the

media every 24 h. After the incubation, cells were fixed by 10% formalin for 60 min followed by washing with iced PBS. Dehydration with 60% isopropanol and stained with Oil Red O solution (3 mg/ml in 60% isopropanol) for 15min at room temperature. After staining, cells were washed with water and then added 100% isopropanol to elute the bound dye for spectrophotometry at 510 nm detection.

MTT assay

Cells were incubated in 33 mM glucose contained media with or without Rutin or EGCG after 2 h starvation. The cell mass was examined by the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay at the indicated duration of time. The MTT working solution (2 mg/ml in PBS) was exposed to each cultured well and incubated for 2-4 h at 37 ℃. The MTT-formazan crystals were dissolved in 1ml of DMSO and the absorbance of 550 nm were performed by spectrophotometer.

Statistical Analysis

All results were expressed as means ± S.D. The significance of difference between experimental groups was performed with Student's *t*-tests. $p < 0.05$ was considered significant. (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$)

Results

Enhancement of glucose stimulated insulin secretion and preservation of the glucose sensing ability during the high glucose incubation in the actions of Rutin or EGCG

The cellular ATP content is responded to the glucose stimulation, and acts as a critical factor to regulate the insulin secretory machinery. Pancreatic β cells were stimulated by 33 mM glucose for 2 h incubation, and this high glucose condition made cells to go through glycolysis to produce ATP. In Fig. 1A, under the high glucose stimulation, cells treated with Rutin or EGCG both effectively elevated the contents of ATP, and that indicated that these two nature occurring compounds might be agents to enhance the efficiency of ATP production in pancreatic β cells. The Correspondences of the insulin secretion also be observed in Fig. 1C, which shown that Rutin and EGCG both increased the insulin to release from cells under high glucose stimulation.

Diabetic individuals might loss the response of glucose stimulation from chronic glucose induced β cell dysfunction, thus to examine whether Rutin or EGCG could still stimulate the insulin secretory machinery of cells that have been long time incubated in high glucose that might be vital for manipulating the β cell function.

Cells were incubated in 33mM glucose contained media for 48 h, and then to detected the cellular ATP content and the released insulin followed by another 2 h high glucose stimulation. In Fig. 1B, Rutin effectively elevated the ATP content, thus EGCG only elevated the ATP content by treating with high dose (10 μM), but not showed significant in low dose treatment (0.1 μM, p=0.224). The insulin secretion of control cells were dramatically suppressed after 48 h high glucose incubation, however, the diminished insulin secretory machinery could be rescue by Rutin or EGCG treatment (Fig. 1D). The actions of Rutin and EGCG might be potent effects to preserve the glucose sensing ability in pancreatic β cells to meet the chronic metabolic demand.

Effects of Rutin or EGCG on the stimulation of IRS2 signaling

The IRS2 signaling in pancreatic β cell regulates the cell survival and maintains the cell function. Pancreatic β cells were stimulated by 33 mM glucose and IRS2 gradually decreased the expression level, nevertheless, cells treated with Rutin or EGCG reversed the effect of high glucose (Fig. 2A). Disruption of IRS2 in mice model has been found that the β cell failure through apoptosis mechanism, and developed to similarities in diabetes, however, Rutin and EGCG both increase the IRS2 protein level against the high glucose incubation.

To determine the action of Rutin and EGCG on IRS2 signaling, the

immunoprecipitation was performed to detect Tyr phosphorylation level of IRS2. When insulin recognized insulin receptor of cell membrane, this bound action would stimulate tyrosine autophosphorylation in the β subunit of receptor, which recruited cellular substrate IRS proteins for Tyr phosphorylation. In Fig. 2B and Fig. 2C, cells were incubated in high glucose condition, cells treated with Rutin or EGCG both elevated the Tyr phosphorylation level after 2 h, furthermore, the effects were also observed after 24 h high glucose incubation. The activation of IRS2 signal would initiate the downstream signal which involved in pancreatic β cell function and proliferation. In Fig. 2D, EGCG rapidly enhanced the FoxO1 phosphorylation level compared with control, and combined Fig.2 C and Fig. 2D results which revealed that EGCG *via* IRS2 activation to increase the FoxO1 nuclear exclusion, however, phosphorylated form Akt was slightly increased from EGCG effect. In this result, the phosphorylation of FoxO1 was induced by high glucose but gradually diminished the phosphorylation after long term high glucose incubation, which could be found in control result; nevertheless, EGCG consistently maintained the phosphorylation rate to make FoxO1 to exclude from nucleus, and that would lead to increase the expression of downstream signal PDX-1.

In Fig. 2E, Rutin enhanced the phosphorylation level of FoxO1 between 6-24 h incubation, comparing with the effect of EGCG, which found that Rutin had less

power to increase and maintain the phosphorylation level of FoxO1. To combined Fig. 2B and Fig. 2E results, which revealed that Rutin could rapidly stimulate the IRS2 signal after 2 h incubation, and then enhance the phosphorylation level of FoxO1 in 6h incubation but the effect would diminish after 24 h incubation, even the phosphorylated form Akt was increased in 12 h incubation. In general, these two natural products both could enhance the IRS2/Akt/FoxO1 signaling under high glucose condition, even EGCG has much powerful effect than the stimulation of Rutin.

Rutin or EGCG Alters PDX-1 Nuclear Translocation and expression

Previous studies have been shown that pancreatic β cell under glucotoxicity condition would suppress the transcription factor PDX-1 activity, which was an essential element for insulin expression level and cell survival. Cells were incubated in high glucose condition, and treated with Rutin or EGCG. In Fig. 3A and Fig. 3B, Rutin and EGCG both effectively enhanced the nuclear translocation activity of PDX-1 after 24 h high glucose incubation. PDX-1 has a crucial role in the determination and differentiation of pancreas, and its transactivation confers the expression of pancreatic β cell specific genes, included insulin, GLUT2 (glucose transporter 2),GK (glucokinase), islet amyloid polypeptide, and another critical

pancreatic transcription factor Nkx 6.1 (22, 41). Rutin and EGCG could promote the PDX-1 nuclear translocation, which mentioned that Rutin and EGCG might be potential agents for glucose sensing and cell survival.

In Fig. 3C, cells were incubated in high glucose condition, and the effect of EGCG elevated the PDX-1 expression level under long term incubation; however, Rutin had no effect on the PDX-1 expression. To combined Fig. 3A and Fig. 3C results, which revealed that Rutin could promote PDX-1 translocation to nuclei, thus the expression level was not influenced. EGCG could enhance the ability of PDX-1 translocation against high glucose incubation; moreover, it also increased the expression level of PDX-1 (Fig. 3B and Fig. 3C).

Inhibition of Glucolipotoxicity *via* AMPK activation

AMPK act as a cellular energy sensor and dysregulation of its signaling network is considered as a key event in causing multiple abnormalities of metabolism (42). Cells were incubated in high glucose condition, and treated with Rutin or EGCG. In Fig. 4A and Fig. 4B, the activity of AMPK was rapidly increased by the treatment of Rutin or EGCG *via* Thr phosphorylation, furthermore, these effects could prolong to 72 h. The reciprocal effect was observed in FAS (Fatty acid synthase) expression, in which the enhancement of AMPK activity by Rutin or EGCG might lead to suppress

the cellular lipogenesis.

The Rutin or EGCG effects of AMPK activity were mediated by upstream signal LKB1 (Fig. 4C and Fig. 4D), following the activated AMPK to downstream signals, which suppress the FAS expression level, in addition, it also negatively regulated the activity of ACC (Acetyl-CoA Carboxylase) through Ser phosphorylation. Pancreatic β Cells elevated the cellular lipogenesis activity under high glucose incubation; however, Rutin and EGCG both effectively suppress the mechanism *via* AMPK signaling network. Long term actions of glucose could disturb the lipid metabolism of β cells (43), nevertheless, Rutin and EGCG might be potential agents for reversing the glucose induced dysregulation of lipogenesis.

The intracellular triglyceride content was correlated to insulin resistance in liver and muscle (44-46), thus the ectopic lipid accumulation in pancreatic β cell might be correlated to cellular dysfunction, even lead to apoptosis (34, 35, 47-49). The cellular lipid accumulation was performed by Oil red o staining, in which cells were incubated in long term high glucose condition for 5 days (Fig. 4E). The treatment of Rutin or EGCG both dramatically suppress the lipid droplets accumulation under high glucose incubation, however, the control cells increased about 43.3% of the cellular fat content to compared with the maintenance glucose condition control. In the Fig. 4 results, both of Rutin and EGCG could provoke the activity of AMPK in pancreatic β

cells under high glucose incubation, and suppress the lipogenesis, eventually effectively decrease the lipid accumulation to delay the toxicity of chronic metabolic stress.

The maintenance of cell mass against chronic hyperglycemic incubation

Hyperglycemic condition could lead to the glucose-induced cell deterioration. The progression of diabetes accompanied with decompensation of pancreatic β cell, which revealed that the preservation of cell mass could be a crucial factor for sustaining the chronic metabolic stress, even coupling with insulin resistance (7, 50-52). Cells were incubated in high glucose condition, and treated with Rutin or EGCG. In Fig. 5A and Fig. 5B, the viable cell mass was determined by MTT assay, and the treatment of Rutin or EGCG both effectively increased the cell viability after 120 h high glucose incubation, however, cells without the treatment led to downregulate the viability. To investigate the mechanism of accelerating proliferation or preserving the cell mass, the Cyclin D1 and anti-apoptotic protein Bcl-2 expression levels were increased in the action of Rutin or EGCG (Fig. 5C and Fig. 5D); furthermore, the pro-apoptotic protein Bax and the Cyclin-dependent kinase (Cdk) inhibitor p21 were successful suppressed by Rutin or EGCG. The effects of Rutin and EGCG in preservation of pancreatic β cell might retain the cell mass against chronic

exposure of hyperglycemic condition.

Long term actions of glucose on pancreatic β cell would lead the occurrence of decompensation through apoptosis mechanism. In Fig. 5E and Fig. 5F, cell cycle pattern was analyzed after long term high glucose incubation, in which the subG1 population gradually increased with the incubation time. The effects of Rutin and EGCG delayed the subG1 phase progression after long term incubation, in other words, these natural occurring compounds could delay the process of decompensation. To determine the underlying mechanism, the western blotting results revealed that Rutin effectively suppressed the p21 expression after long term incubation, furthermore, upregulated Cyclin D1levels (Fig. 5H). In Fig. 5I, Rutin inhibited the glucose induced maturation of SREBP1, which has been considered as a critical factor related to pancreatic β cell dysfunction (53, 54).

The effect of EGCG to protect pancreatic β cell from glucose induced toxicity, in which EGCG slightly increased the expression level of Cyclin D1, and inhibited the p21 accumulation. Moreover, EGCG consistently suppressed the ACC activity *via* Ser79 phosphorylation and the expression of SREBP1 (Fig. 5J). In conclusion, the effects of Rutin and EGCG protect pancreatic β cells from glucotoxicity, in which they suppress p21 accumulation and increase Cyclin D1 expression level to driving the cell cycling, and restrain the intracellular lipogenesis from long term induction of

glucose.

Discussion

The prevalence of diabetes is estimated to be 2.8% in 2000, and predicted that could be 4.4% in 2030. Based on the global survey of diabetes implicated the devastating difference of the prevalence of diabetes between urban and rural populations in developing countries, which revealed that the association of diet alteration, stress, obesity, and decreased physical capacity (55). To resolve this modern disease, several drugs have been developed to treat diabetes; however, the complicated disorder of metabolism has no complete cure yet (56-58). Tea is a common traditional beverage in ordinary life of Asian people, and previous studies have suggested the novel function in anti-diabetes and anti-obesity from its ingredients. EGCG, most abundant tea polyphenol in green tea, has been reported in attenuation of insulin signaling and insulin mimetic effect (38, 59, 60). Furthermore, buckwheat, a natural herb, has been demonstrated the ability to reduce blood glucose (39), and improvement of glucose homeostasis from its ingredient called Rutin. Natural occurring compounds are easy to obtain and have lower price to chemical drugs, hence, the advantages of natural products might let them to be novel agents for the treatment of diabetes.

Patients with the metabolism syndrome manifest complex abnormalities of

glucose and lipid metabolism, and gradually develop to interference of multiple tissues (61-63). Excess energy intake and decreased exercise capacity would lead to dysregulate the metabolic harmony, which implicate to increase the rates of obesity-associated disease, such as type 2 diabetes. The etiological factors of type 2 diabetes are typical insulin resistance, and accompanied with pancreatic β cell deterioration, in which previous studies have demonstrated the crucial role of pancreatic β cell in the pathogenesis of diabetes to seize the debate of its contribution (7, 10, 64).

Pancreatic β cell is a fundamental element for the development of diabetes, and its unique secretory capacity of insulin manipulates individual energy balance. Impairment of glucose-stimulated insulin secretion (GSIS) might cause from the deadaptation of peripheral insulin resistance or chronic exposure of high glucose condition. Chronic overloaded metabolic demand crashes β cell in response to glucose, which could lead to a progressive deterioration of cell function (65). Once glucose transporters facilitate the glucose entry to pancreatic β cell, and then the glycolysis process responses to generate ATP considered as a factor in secretory mechanism. Previous studies have shown that mitochondrial dysfunction linked to β cell dysregulation (66, 67), hence in the Fig. 1 results, Rutin and EGCG has the effect on elevation of cellular ATP production under short-tem glucose stimulation or

incubation of long-term high glucose condition. The maintenance of ATP productive efficiency revealed the preservation of glucose sensing ability by the actions of Rutin and EGCG. Long term actions of glucose dramatically decrease the insulin secretory capacity to cause the insulin insufficiency; nevertheless, Rutin and EGCG both effectively increase the secretory activity in response to glucose stimulation, even after the long-term high glucose incubation.

The insulin secretory machinery in glucose-stimulated response, leading to activate the IRS2 signaling through the autocrine mechanism. IRS2 signaling controls the function and proliferation of pancreatic β cell, thus disrupted the pathway could lead to lose the insulin action and cell failure. Rutin and EGCG have the effect on enhancement of insulin secretion, furthermore, they increase the activity of IRS2 pathway (Fig.2). Previous studies have shown the role of IRS2 in diabetes (14, 16, 68), the effect of Rutin and EGCG to increase the IRS2 expression level and activate its signaling *via* Tyr phosphorylation, which might attenuate the high glucose-induced toxicity.

The major downstream signaling of IRS2 is Akt/FoxO1, in which Akt has been reported involved in pancreatic β cell proliferation and insulin secretion ability (18, 57, 69, 70). The effects of Rutin and EGCG on enhance the Akt activation are observed in 6-12 h, however, FoxO1 is rapidly phosphorylated before the activated Akt

enhancement. FoxO1 as a critical factor in insulin-regulated gene expression, such as inhibition of hepatic glucose production *via* suppress the expression of PEPCK, and repression the transcription factor PDX-1 expression in pancreatic β cell. Phosphorylated FoxO1 is excluded from nucleus that has been suggested to involve in pancreatic β cell compensation to peripheral insulin resistance (21). The actions of Rutin and EGCG have the improvement of Akt activity, and FoxO1 nuclear exclusion *via* Ser phosphorylation, however, FoxO1 is excessive phosphorylated earlier then the effect on Akt, retaining other signalings involved in this mechanism, such as MEK/ERK signaling.

The treatment of Rutin or EGCG could increase and preserve the glucose sensing ability of pancreatic β cell, then enhance the insulin secretory machinery in response to glucose stimulation. The efficient secreted insulin has much effect on activation of IRS2 signaling, following to deliver it to the downstream signals which maintain the cell function and viability. PDX-1 is a vital transcription factor in β cells which regulates the expression levels of insulin, GLUT2, GK, and its function is affected by the cellular localization (23, 71). Glucotoxicity or lipotoxicity would lead to reduce the expression of PDX-1 or block the nuclear translocation, however, the treatment of Rutin or EGCG both promoted the nuclear translocation under high glucose incubation, which could lead to activate its regulation of responsive genes expression.

The effect on PDX-1 from Rutin and EGCG is diverse in its expression level. The difference between Rutin and EGCG to alter the expression, which could correlate with the effect on IRS2 signaling, in which EGCG could stimulate the IRS2/Akt/FoxO1 signaling better than Rutin did.

The synergism of disorder of glucose and lipid metabolism would accelerate the deterioration of pancreatic β cell, which could result from long term action of glucose. Accumulation of lipids in pancreatic β cell have been shown to precede the onset of dysfunction, and even associated with insulin resistance in other tissues, such as liver and muscle (72, 73). Profound effects of declined AMPK activity not only involved in fuel sensing ability, but have been considered to link with insulin resistance and upregulate the cellular lipogenesis. Several developed anti-diabetic drugs, such as metformin and TZDs (Thiazolidinediones), have been proved to activate AMPK and effectively treat insulin resistance and disorders associated with the metabolic syndrome.

Rutin and EGCG act as activators of AMPK and rapidly enhance the activity *via* Thr phosphorylation against high glucose incubation, which presents a reciprocal effect on FAS expression. Chronic elevated glucose could initiate the activation of cellular lipogenesis, in which lipogenic genes expression would be upregulation. The effects on AMPK activity of Rutin and EGCG suppressed the FAS expression, and

depressed the activity of ACC *via* Ser phosphorylation; moreover, they interrupted the SREBP1 maturation or generation in causing the inhibition of lipid biogenesis (Fig.4). AMPK activation protects cells against insulin resistance and cellular dysfunction in regulation of energy homeostasis; therefore Rutin and EGCG play crucial roles in maintaining the manners of glucose and lipid metabolism caused from chronic exposure of high glucose condition in pancreatic β cell.

SREBP1, a lipogenic transcription factor, has been shown as a instrumental factor in the development of pancreatic β cell dysfunction (53). Overexpression of SREBP1 would lead to β cell dysfunction, which increases the lipogenic gene expression, furthermore, it targets multiple genes implicated in cell growth and survival. After adaptation of insulin resistance, pancreatic β cell goes through the decompensation process and into the onset of progressive deterioration of cell function, and massive cell failure in causing apoptosis. Induction of SREBP1 would cause defects in genes implicated in glucose metabolism and cellular function, such as GLUT2, glucokinase, PDX-1, IRS2, p21, and BAX. IRS2 and PDX-1 as important factors to maintain β cells growth and survival, which could be suppress the expression *via* SREBP1 overexpression, however, p21 and BAX would be elevated in provoking cell cycle arrest and facilitating apoptosis to occur (74).

The impact of Rutin and EGCG on pancreatic β cell would suppress the

elevating expression of p21 and Bax with the high glucose incubation. To meet the chronic overloaded metabolic demand, the cell mass is essential for preservation of insulin sufficiency in response of glucose action. Cyclin D1 and Bcl-2 are regard as critical factors in cell proliferation and expansion (75-78), therefore to effective maintain the cell population could be argument against decompensation (Fig.5). The effects of Rutin and EGCG affected the cell viability *via* the expression levels of Cyclin D1 and Bcl-2 under high glucose incubation, which implicated that Rutin and EGCG could prolong the adaptation to delay the onset of progression.

Type 2 diabetes is caused from multiple abnormalities of metabolism, in order to find out the cure candidate, scientists have investigated the underlying molecular mechanism for decades. Natural herbs contain countless chemical compounds that have been used in traditional Chinese medicine provided abundant sources for validation (79, 80). The tea polyphenol EGCG and the citrus flavonoid glycoside Rutin in this study have been observed the effects on pancreatic β cell whether they could be candidates for improving cell function and viability against chronic metabolic demand. Broad effects of Rutin and EGCG are elucidated the roles in manipulating the glucose and lipid metabolism on β cells, which effectively keep the order of energy homeostasis under long term actions of glucose. The current study highlights the novel function of two natural occurring compounds called Rutin and

EGCG might be agents for protection of pancreatic β cell against glucotoxicity.

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Figures

Figure 1. Enhancement of Glucose Stimulated Insulin Secretion and preservation of glucose sensing ability during the high glucose incubation in the action of Rutin or EGCG

Fig. 1A

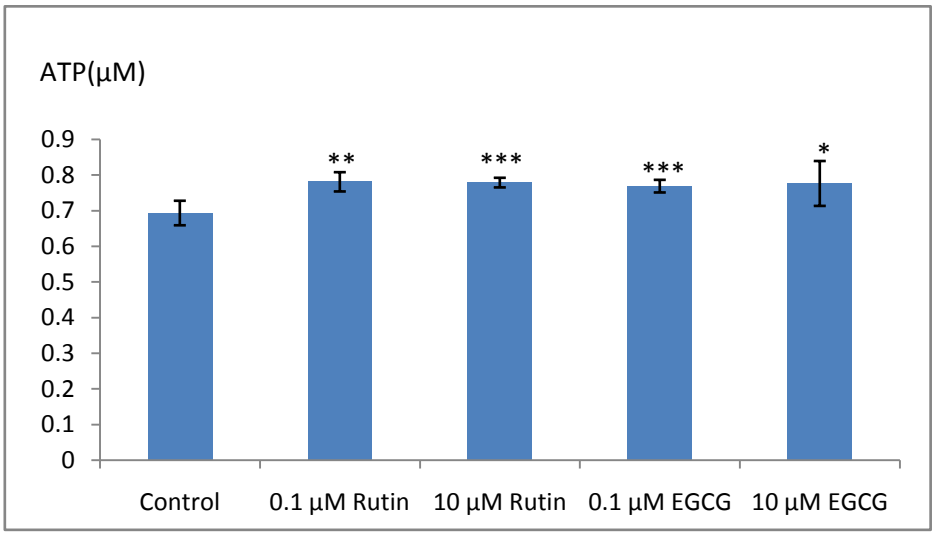


Fig. 1B

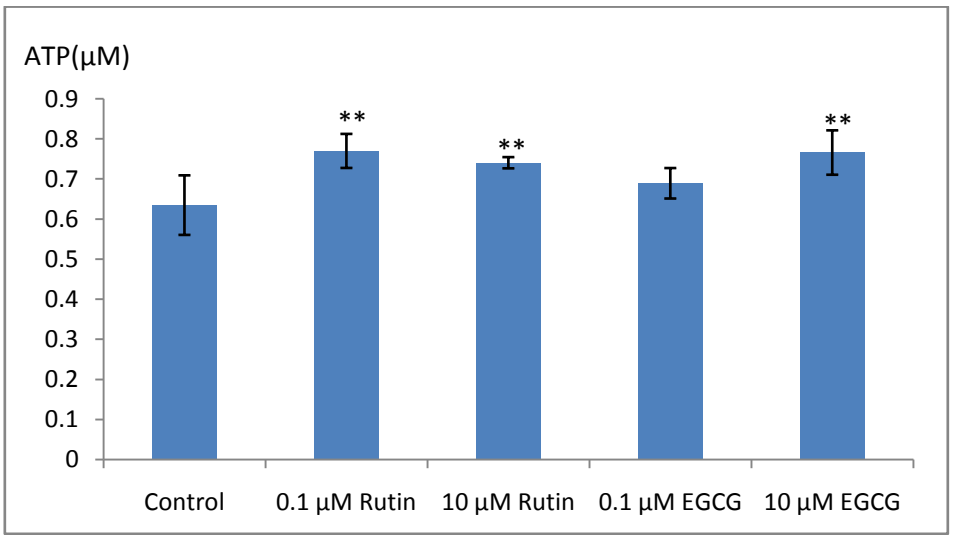


Fig. 1C

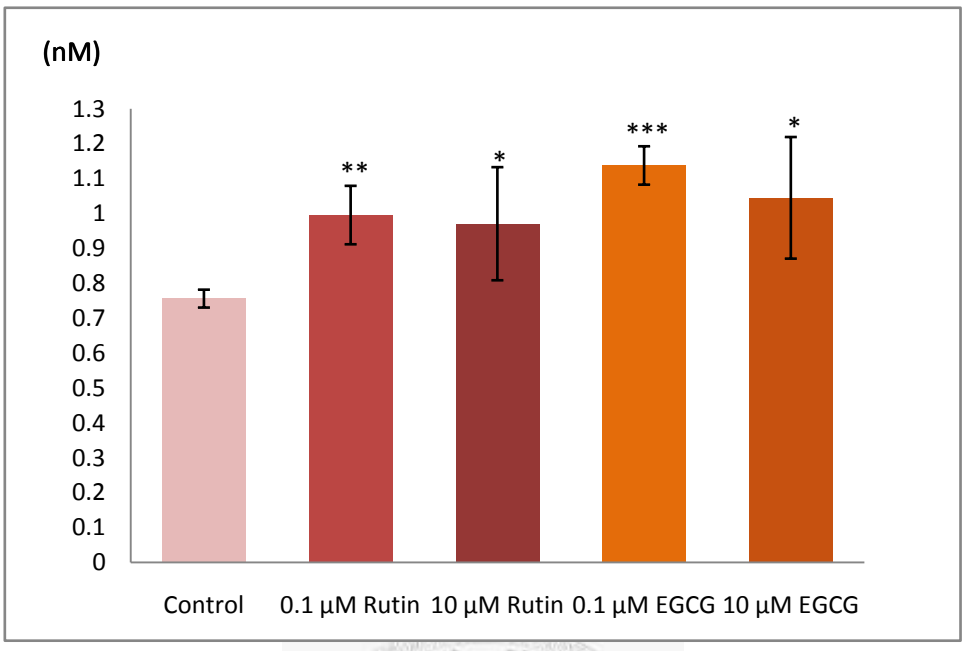

(nM)
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
**
*
***
*
Control
0.1 μM Rutin
10 μM Rutin
0.1 μM EGCG
10 μM EGCG

Fig. 1D

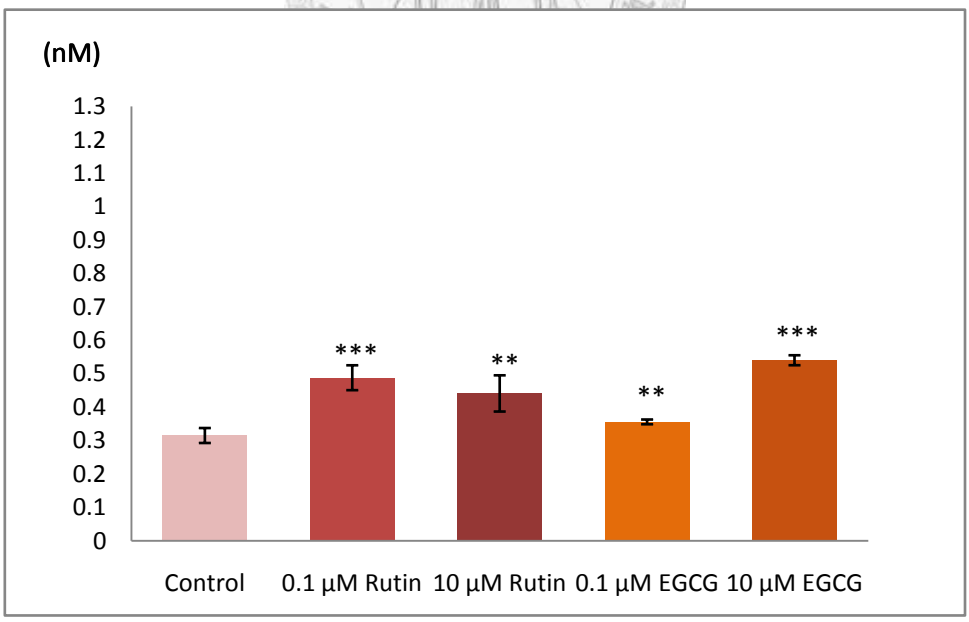

(nM)
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
***
**
**
***
Control
0.1 μM Rutin
10 μM Rutin
0.1 μM EGCG
10 μM EGCG

Fig. 1A, B| (A)The cellular ATP contents were detected after the 2 h stimulation of 33 mM glucose, and treated with Rutin or EGCG that both effectively increased the ATP contents compared to the control (B) The cells were incubated in 33 mM glucose contained medium for 48 h (chronic high glucose incubation), then replaced the high glucose medium w/o Rutin or EGCG. The cellular ATP contents were observed to increase in the treatment of Rutin or EGCG.

Fig. 1C, D| (C) The insulin secretion was effectively increased in the action of Rutin or EGCG after the high glucose stimulation (2 h) (D) Rutin or EGCG both could preserve the ability of glucose sensing after chronic high glucose incubation (48 h), and leads to effectively secrete the insulin.

Figure 2. Effects of Rutin or EGCG on the stimulation of IRS2 signaling

Fig. 2A

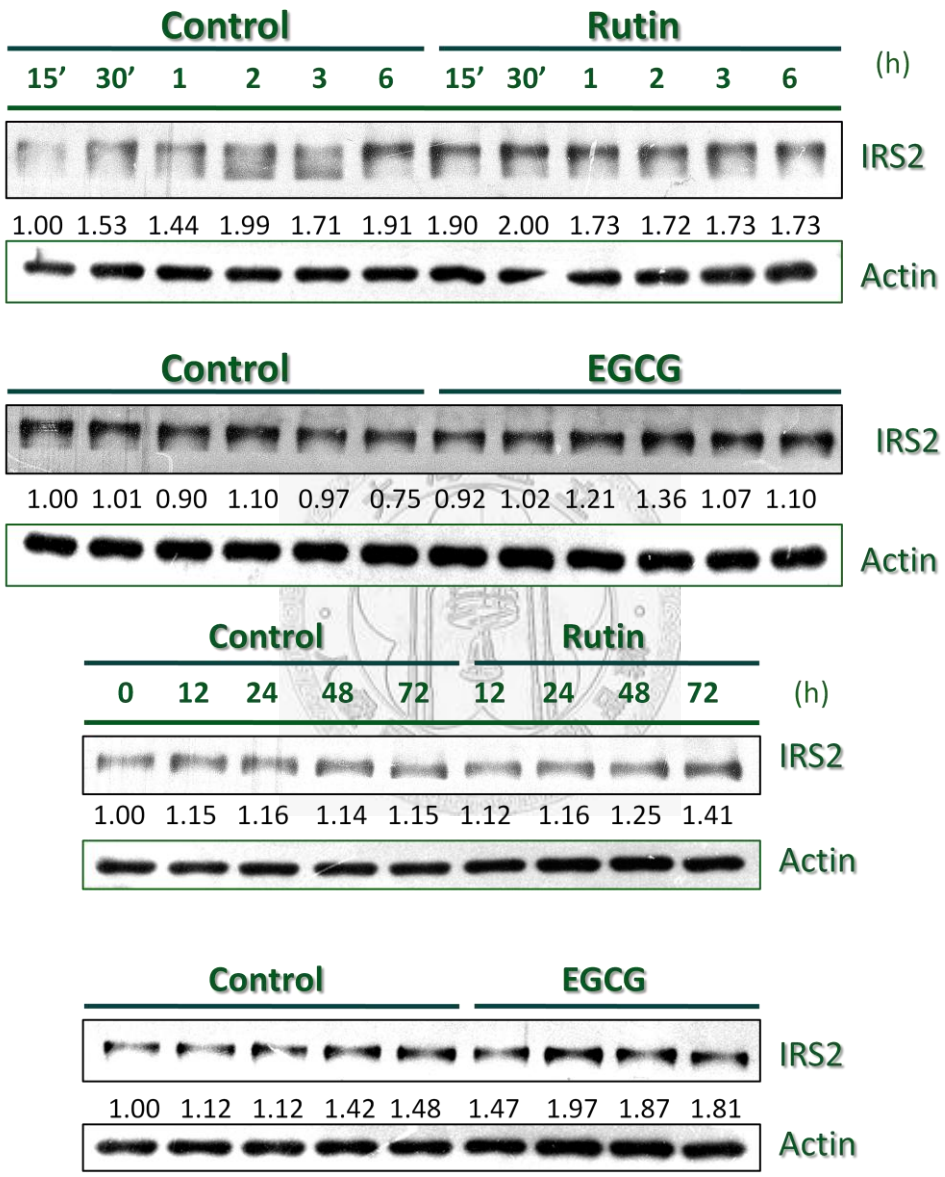


Fig. 2A| Cells were incubated in high glucose condition and treated with 10 μM Rutin or 10 μM EGCG. After treatment, Rutin and EGCG both maintain and slightly promote the expression of IRS2 correlated to the control in the high glucose incubation.

Fig. 2B

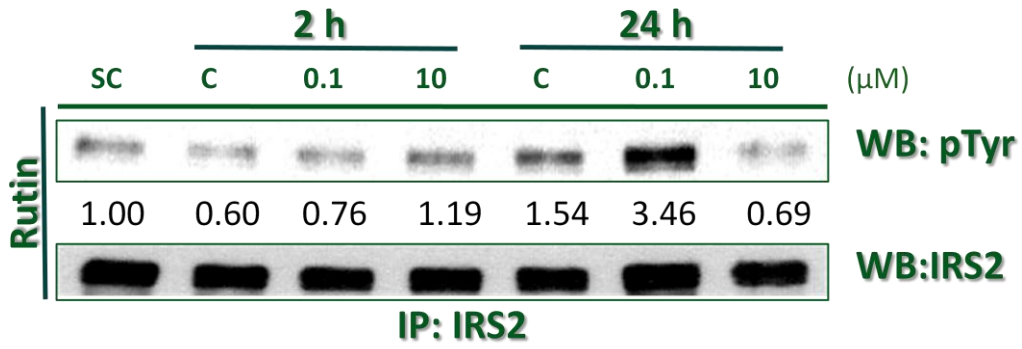


Fig. 2C

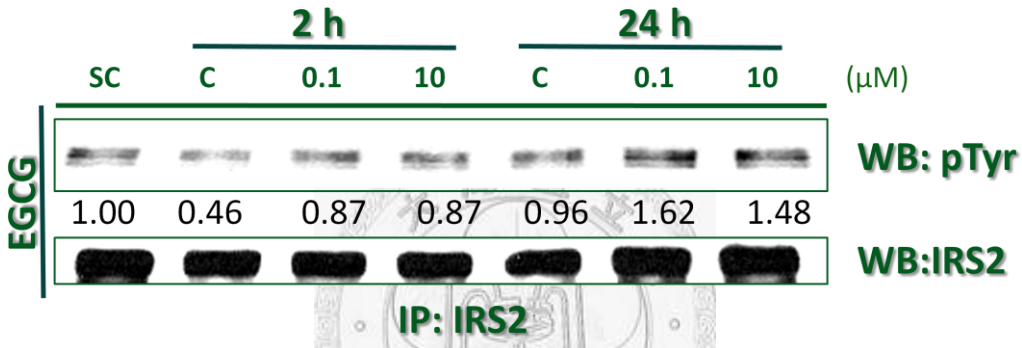


Fig. 2B, C| The IRS2 signaling activity was observed by Tyr phosphorylation through immunoprecipitation. Cells were incubated in high glucose condition treated with 10 μM Rutin or 10 μM EGCG. The blotting analysis was performed and found that Rutin and EGCG both enhanced the IRS2 activation *via* Tyr phosphorylation after 2/24 h high glucose incubation. (sc=starvation control, c=33 mM glucose condition control)

Fig. 2D

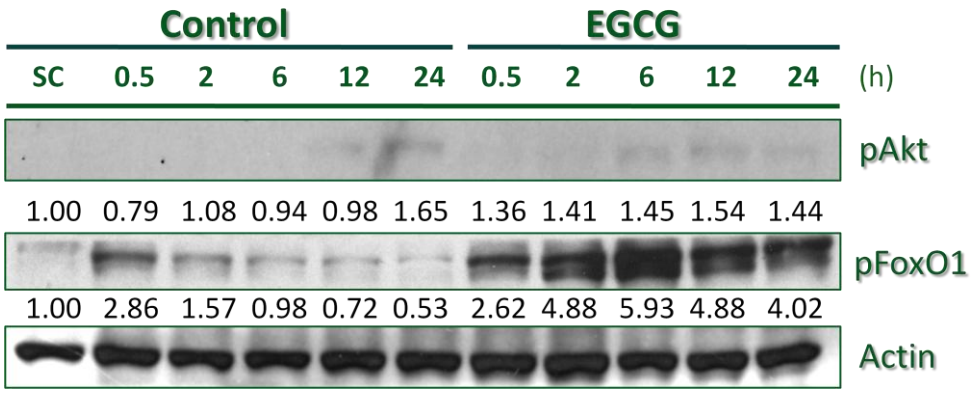


Fig. 2E

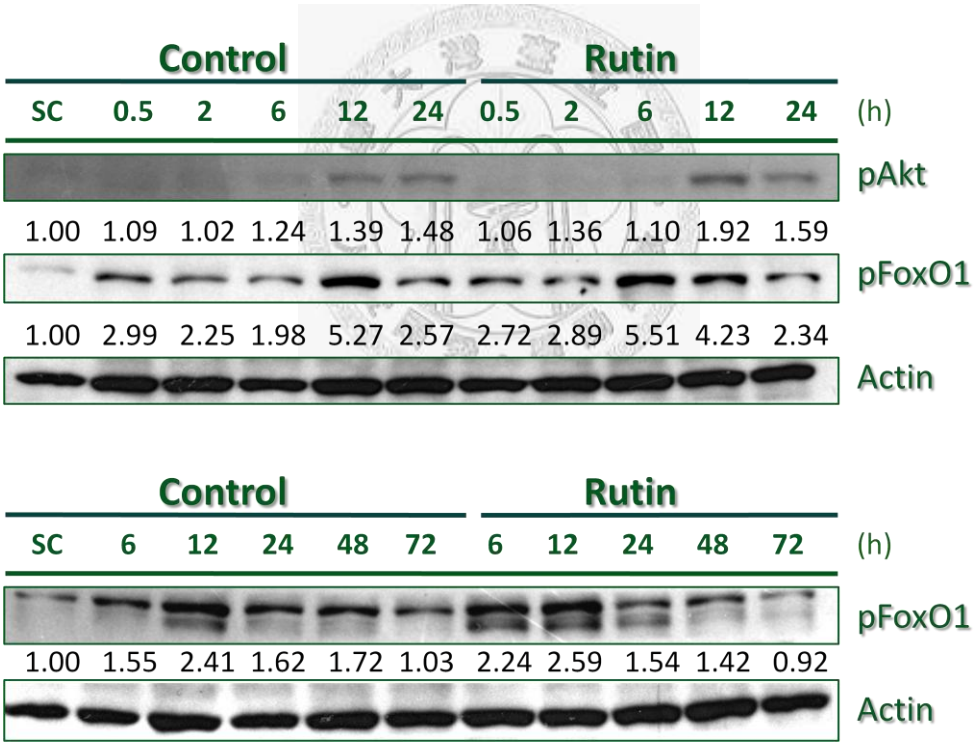


Fig. 2D, E| EGCG or Rutin effectively phosphorylated FoxO1 to cause the nuclear exclusion upon the IRS-2/Akt activation. In contrast with EGCG treatment, Rutin took much longer time to phosphorylate FoxO1.

Figure 3 Rutin or EGCG Alters PDX-1 Nuclear Translocation and expression

Fig. 3A

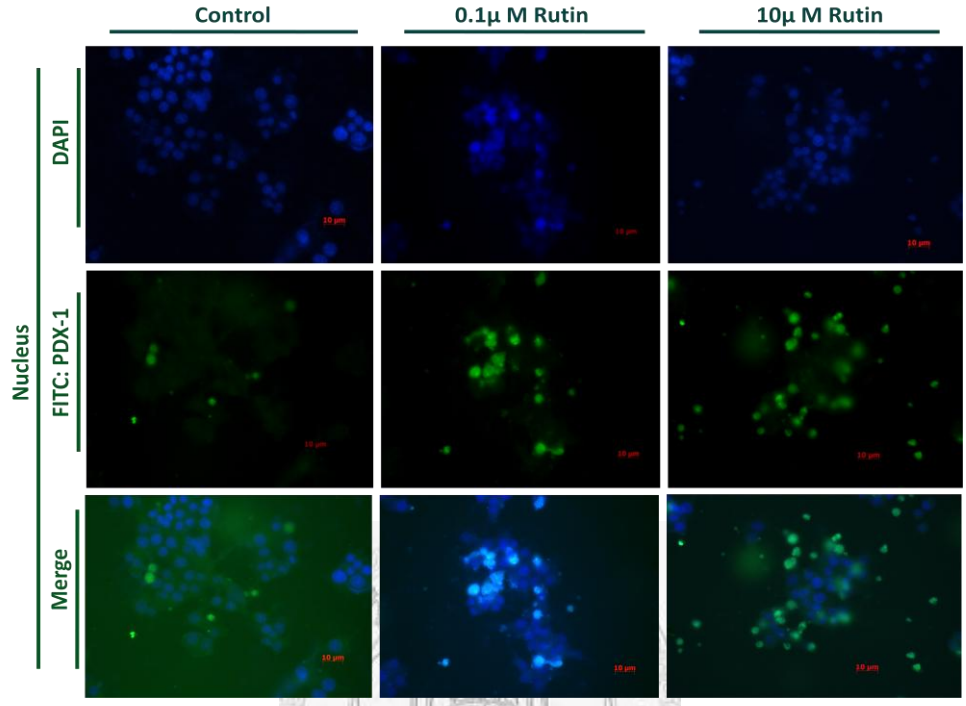


Fig. 3B

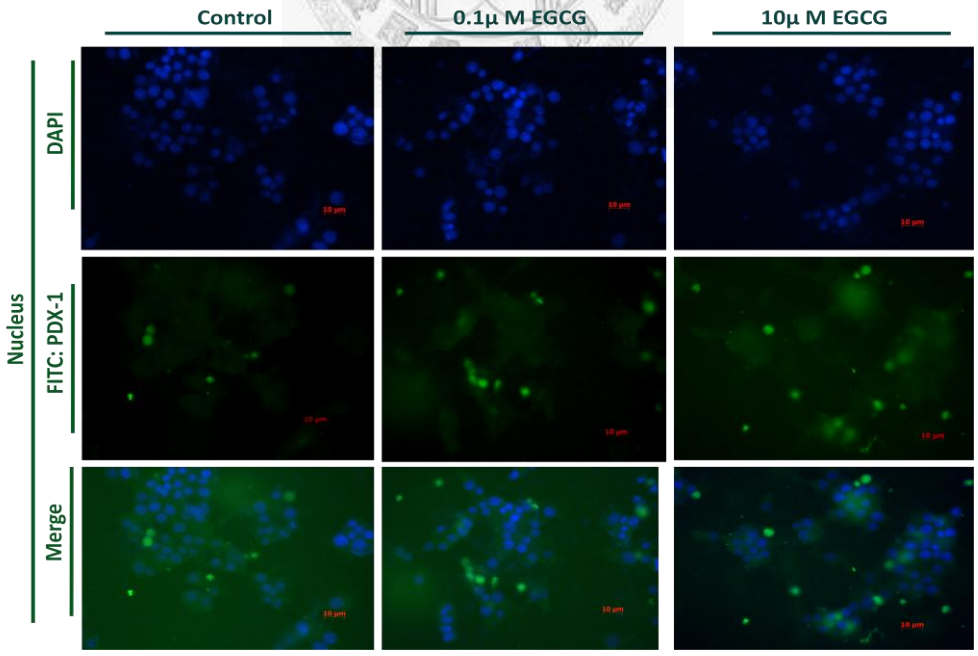


Fig. 3C

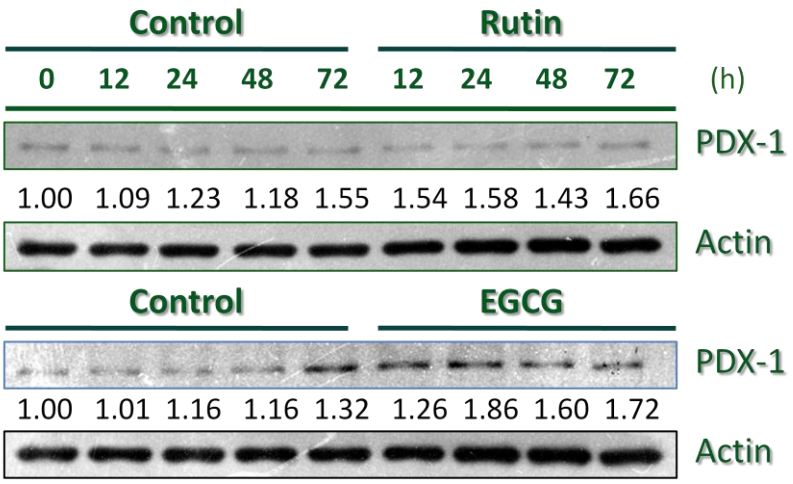


Fig. 3A, B| Cells were incubated in high glucose condition for 24 h. Comparing with high glucose control, Rutin and EGCG both promoted PDX-1 to process the nuclear translocation.

Fig. 3C| Under the high glucose incubation, 10 μM EGCG substantially increased the expression of PDX-1. In contrast with EGCG treatment, Rutin had less effect on the PDX-1 expression.

Figure 4 Inhibition of Glucolipotoxicity *via* AMPK activation

Fig. 4A

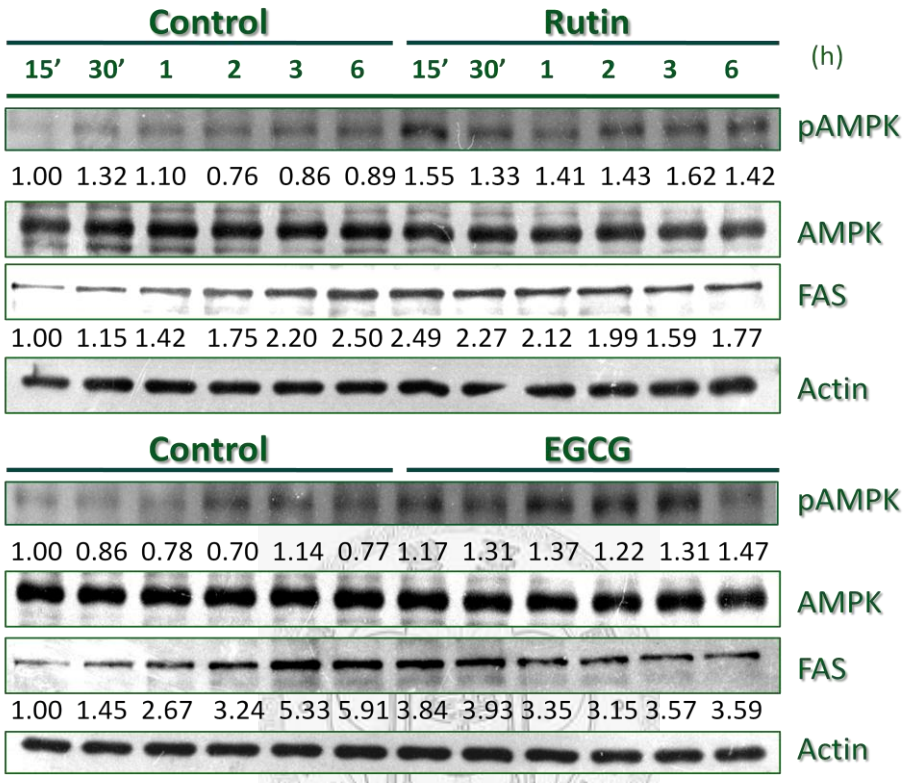


Fig. 4B

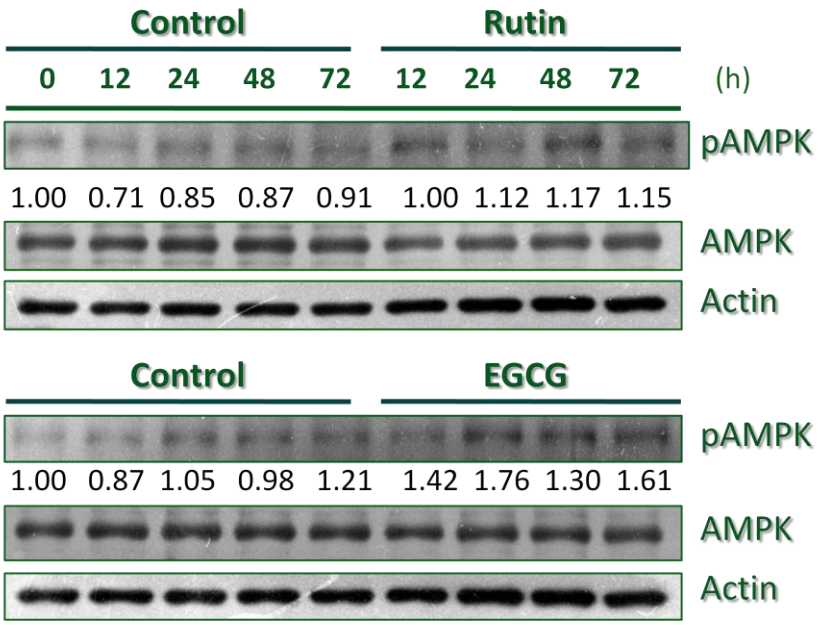


Fig. 4C

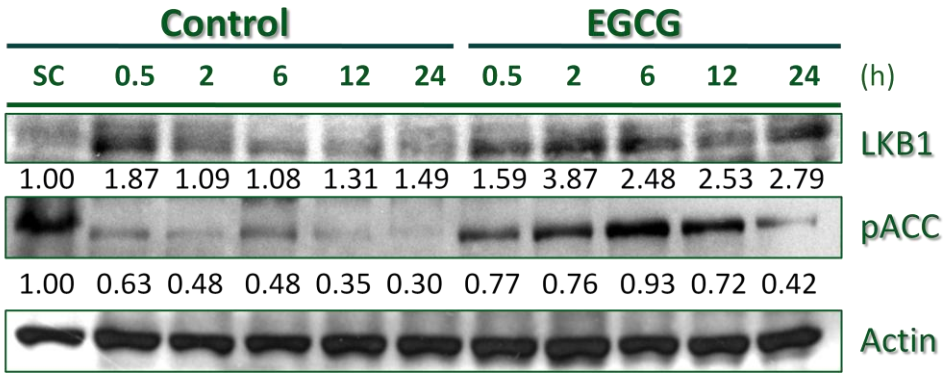


Fig. 4D

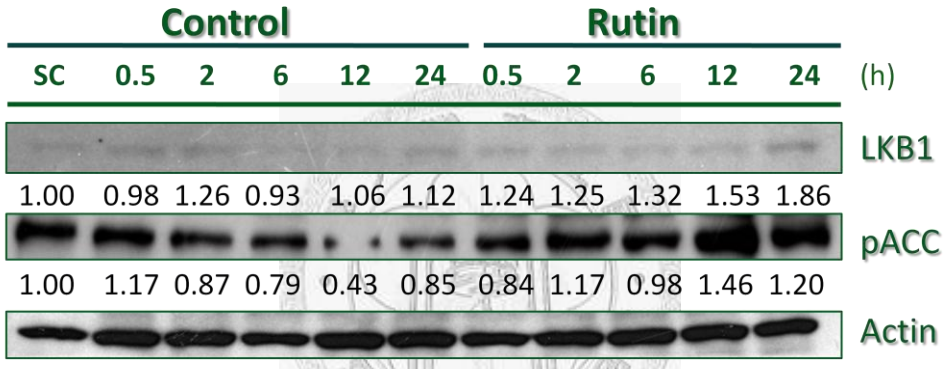


Fig. 4A, B| Cells were incubated in high glucose condition and treated with 10 μM Rutin or 10 μM EGCG. The high glucose incubation suppressed AMPK activity and increased the FAS expression with incubation period. Rutin and EGCG both reversed the AMPK activity in the high glucose incubation and led to suppress the FAS expression.

Fig. 4C, D | ECGC or Rutin activated AMPK by the mediation of LKB1 and led to suppress the activity of ACC *via* phosphorylation.

Fig. 4E

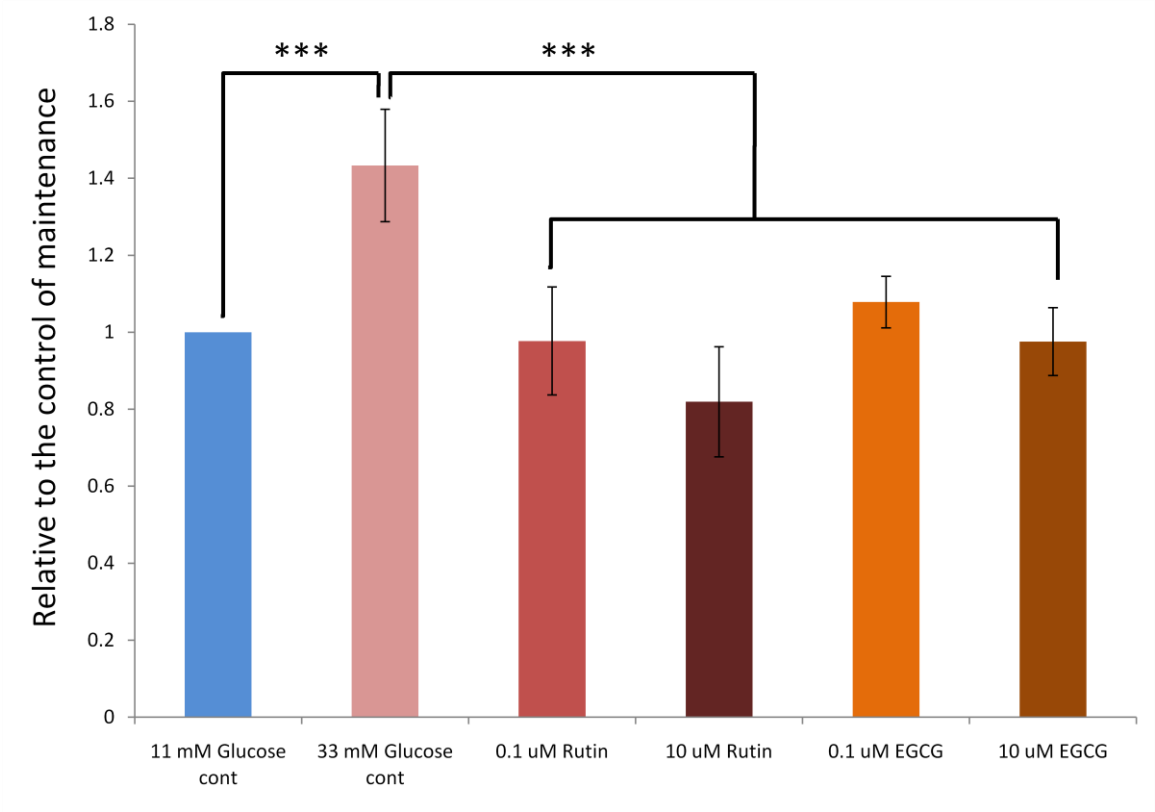


Fig. 4E| After the chronic high glucose incubation (5 days/ 33 mM glucose), the cells accumulated lipid droplets by the metabolic stress. However, cells treated with Rutin or EGCG both effectively reduced the lipid accumulation and down to the level of maintenance glucose control (11 mM glucose).

Figure 5. The maintenance of cell mass against chronic hyperglycemic incubation

Fig. 5A

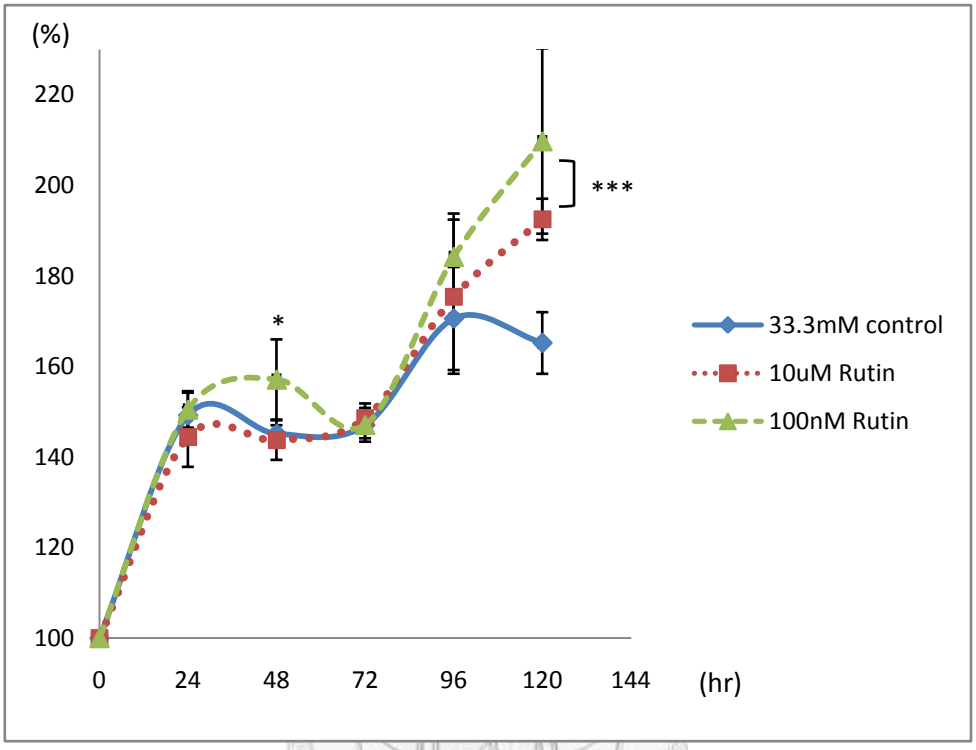


Fig. 5B

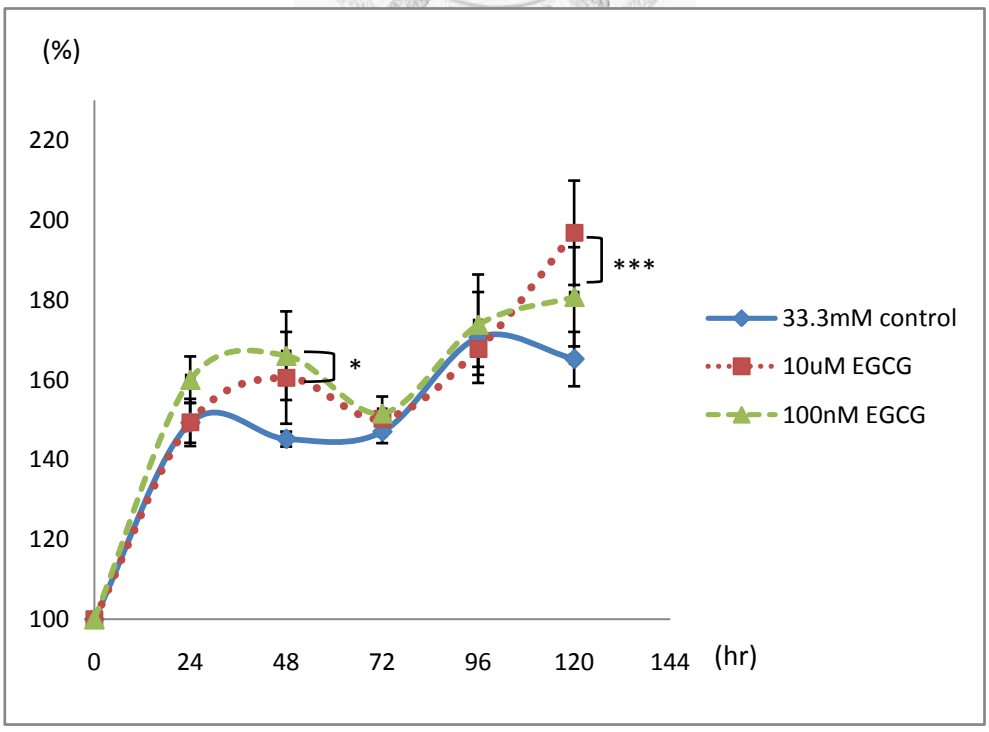


Fig. 5C

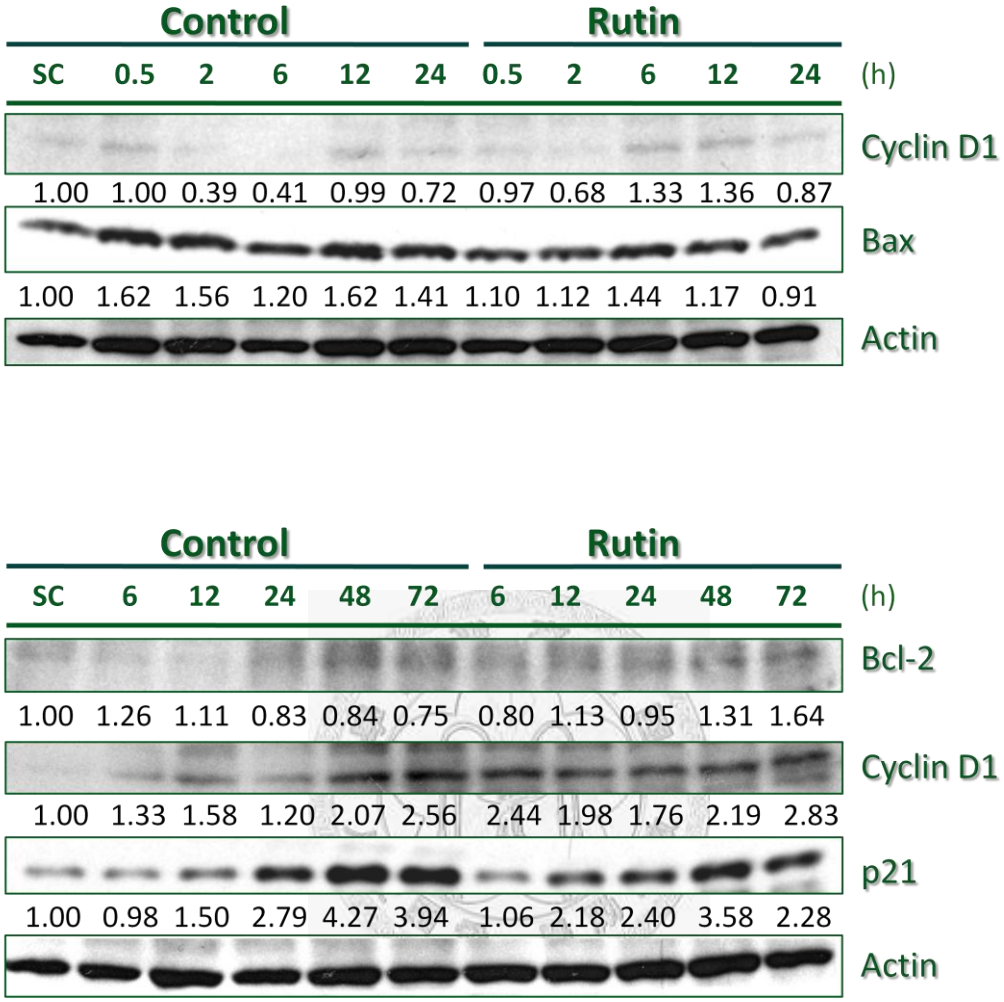

Control
Rutin
SC 0.5 2 6 12 24 0.5 2 6 12 24 (h)
Cyclin D1
1.00 1.00 0.39 0.41 0.99 0.72 0.97 0.68 1.33 1.36 0.87
Bax
1.00 1.62 1.56 1.20 1.62 1.41 1.10 1.12 1.44 1.17 0.91
Actin
Control
Rutin
SC 6 12 24 48 72 6 12 24 48 72 (h)
Bcl-2
1.00 1.26 1.11 0.83 0.84 0.75 0.80 1.13 0.95 1.31 1.64
Cyclin D1
1.00 1.33 1.58 1.20 2.07 2.56 2.44 1.98 1.76 2.19 2.83
p21
1.00 0.98 1.50 2.79 4.27 3.94 1.06 2.18 2.40 3.58 2.28
Actin

Fig. 5D

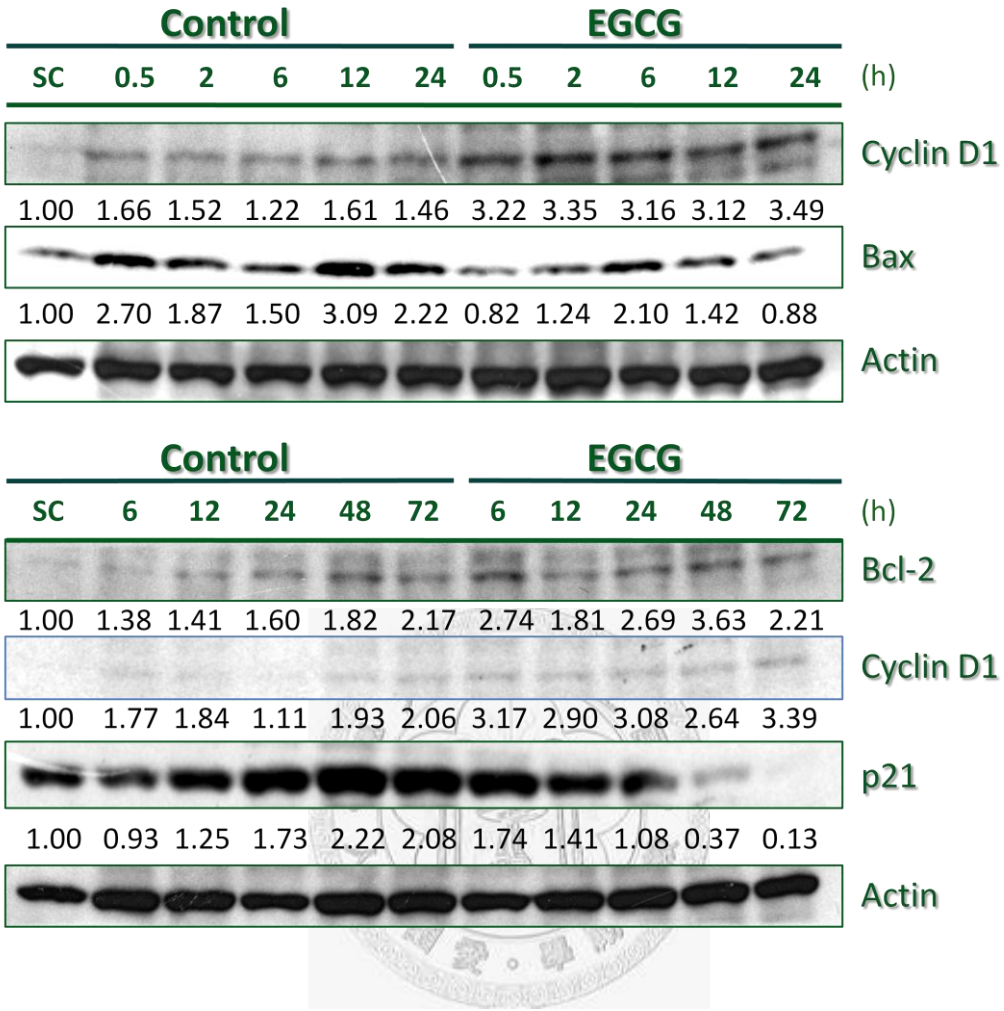


Fig. 5A, B| Cells were incubated in chronic high glucose condition with or without Rutin or EGCG treatment. Cell mass was performed by MTT assay, which revealed that Rutin or EGCG preserved and effectively increased cell mass after the high glucose incubation (120 h).

Fig. 5C, D| Cells were incubated in high glucose condition. Rutin or EGCG both suppressed p21 and Bax expression. In the other hand, they increased Cyclin D1 and

the anti-apoptotic protein Bcl-2 expression in the high glucose incubation.

Fig. 5E

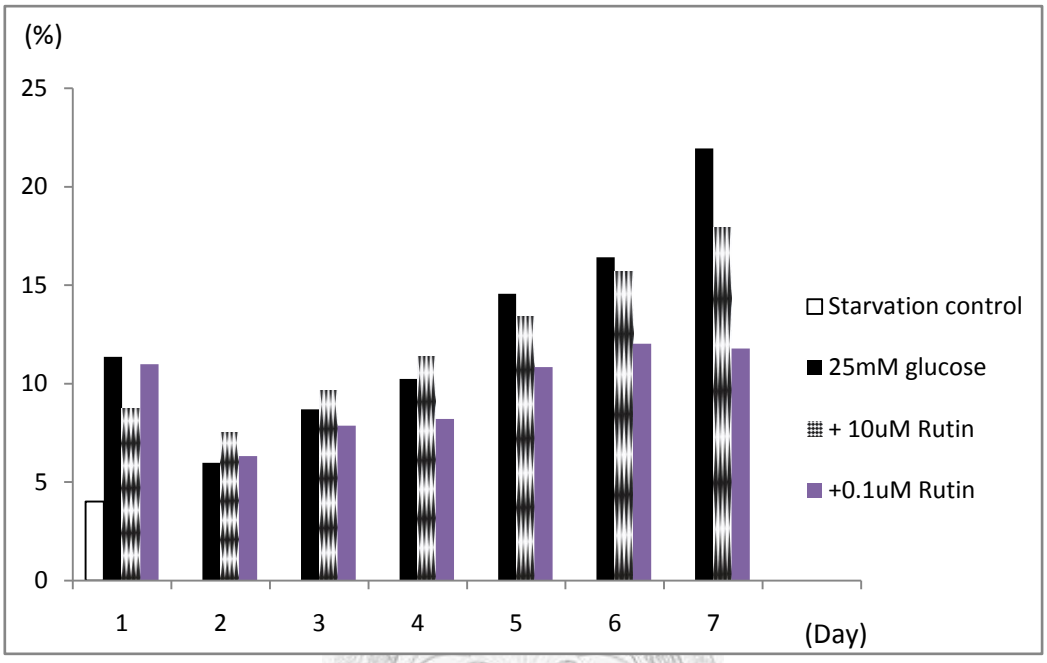


Fig. 5F

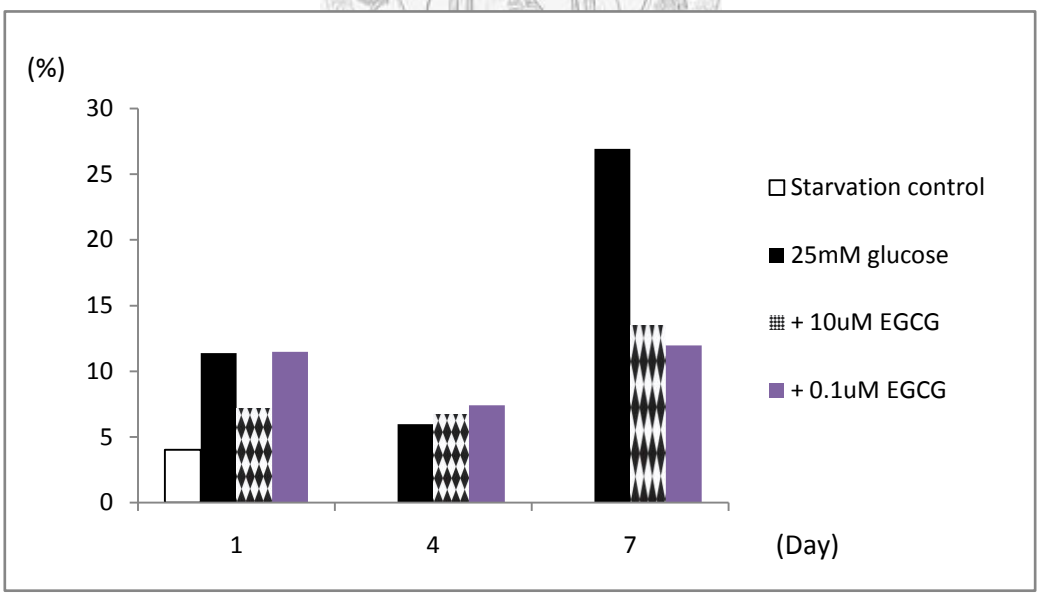


Fig. 5E, F| Rutin or EGCG could effectively suppress the subG1 phase to arise from the chronic hyperglycemic incubation.

Fig. 5G

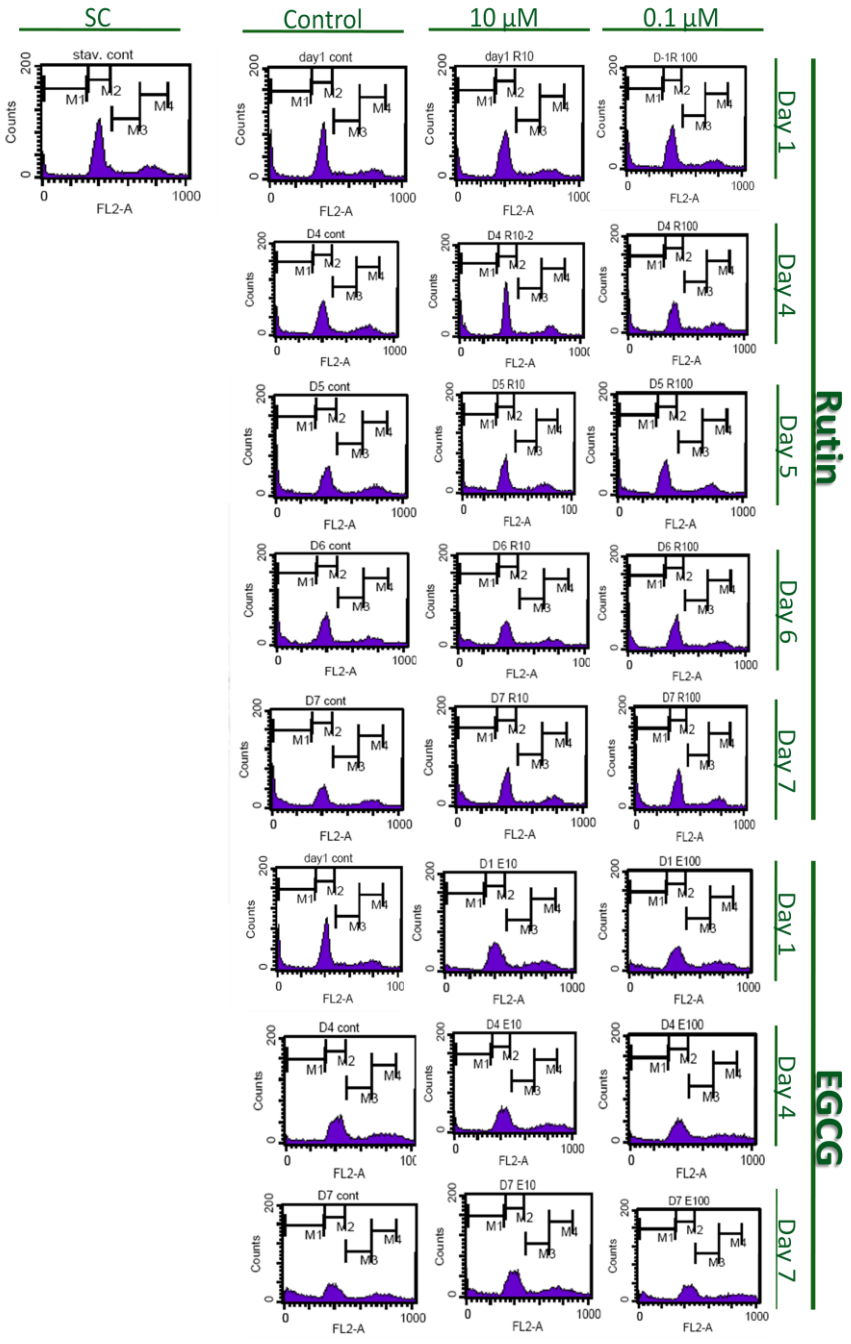


Fig. 5G| The cell cycle pattern form incubation of 25 mM contained media with or without Rutin or EGCG treatment. (sc= starvation control)

Fig. 5H

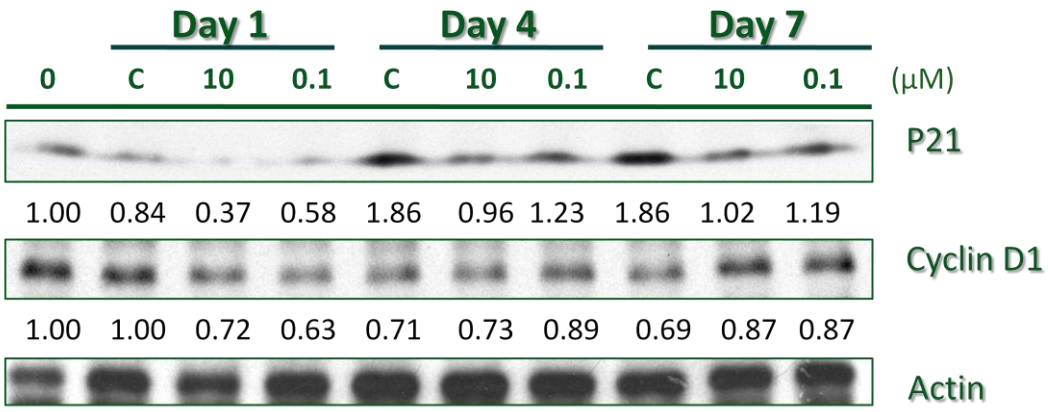


Fig. 5I

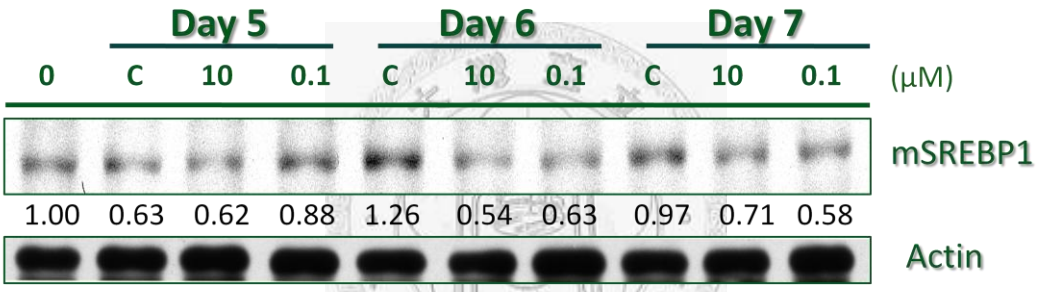


Fig. 5G, H| Rutin suppressed the accumulation of p21 and the maturation of SREBP1, in the other hand, Rutin also could increase the expression of Cyclin D1. (mSREBP1= mature form of SREPB1)

Fig. 5J

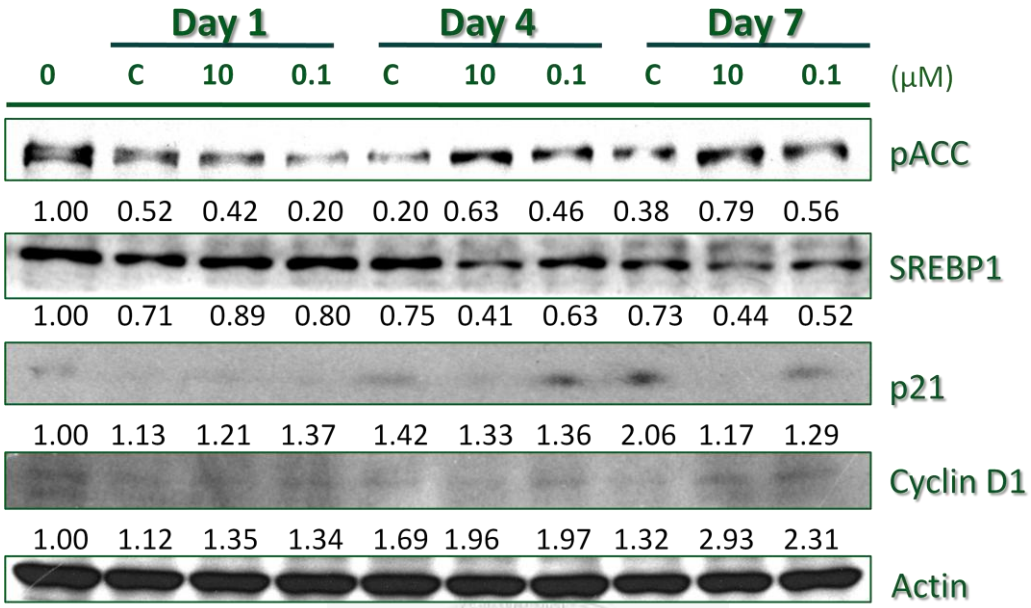
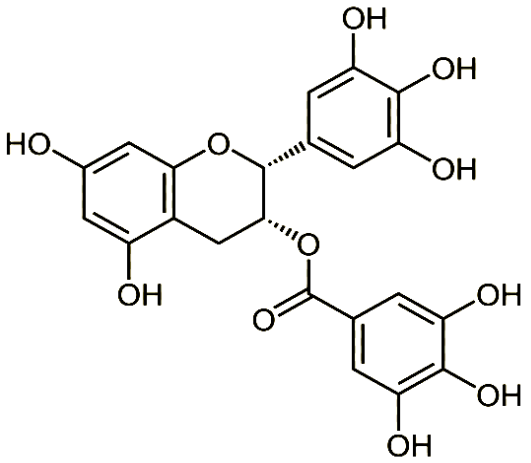
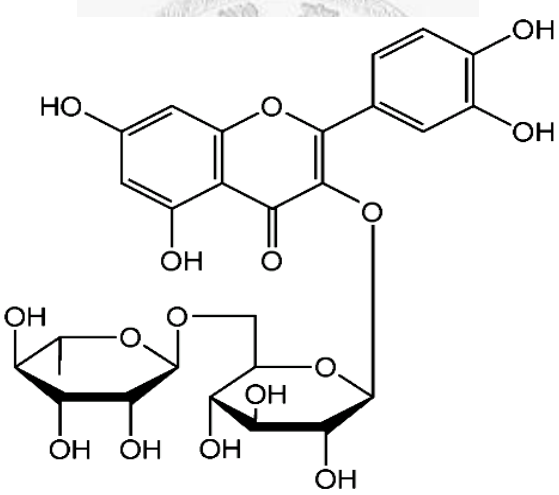


Fig. 5I| EGCG suppressed the expression of SREBP1 and p21, furthermore, increased the Cyclin D1 expression and inhibited ACC activity *via* Ser79 phosphorylation.

Appendices

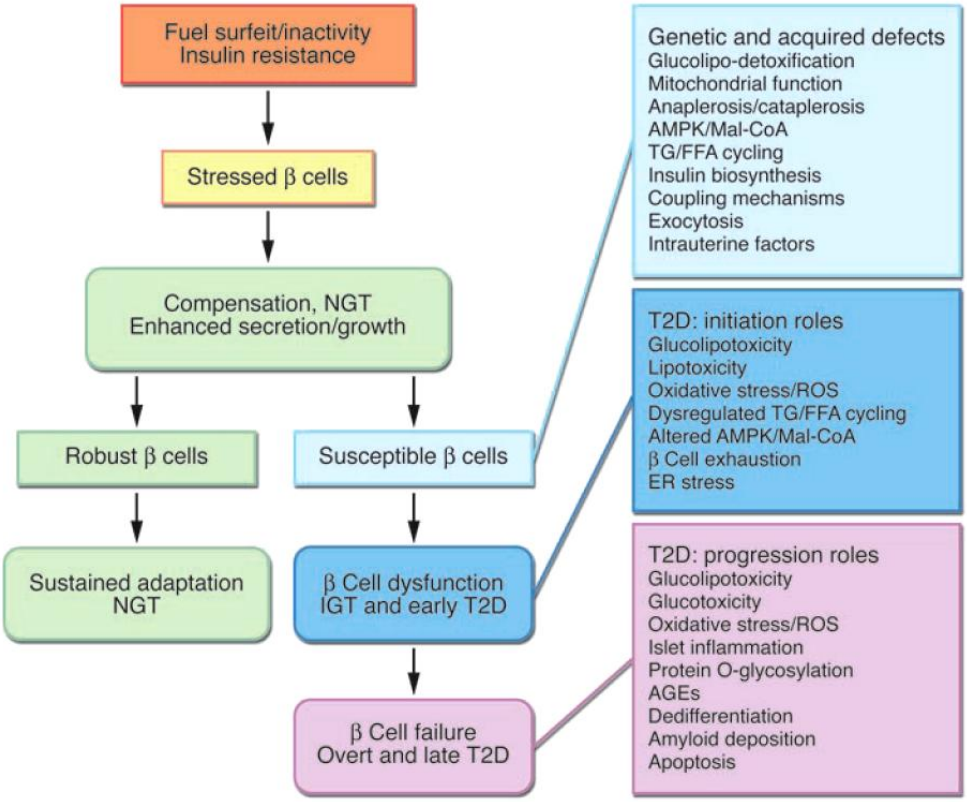


EGCG



Rutin

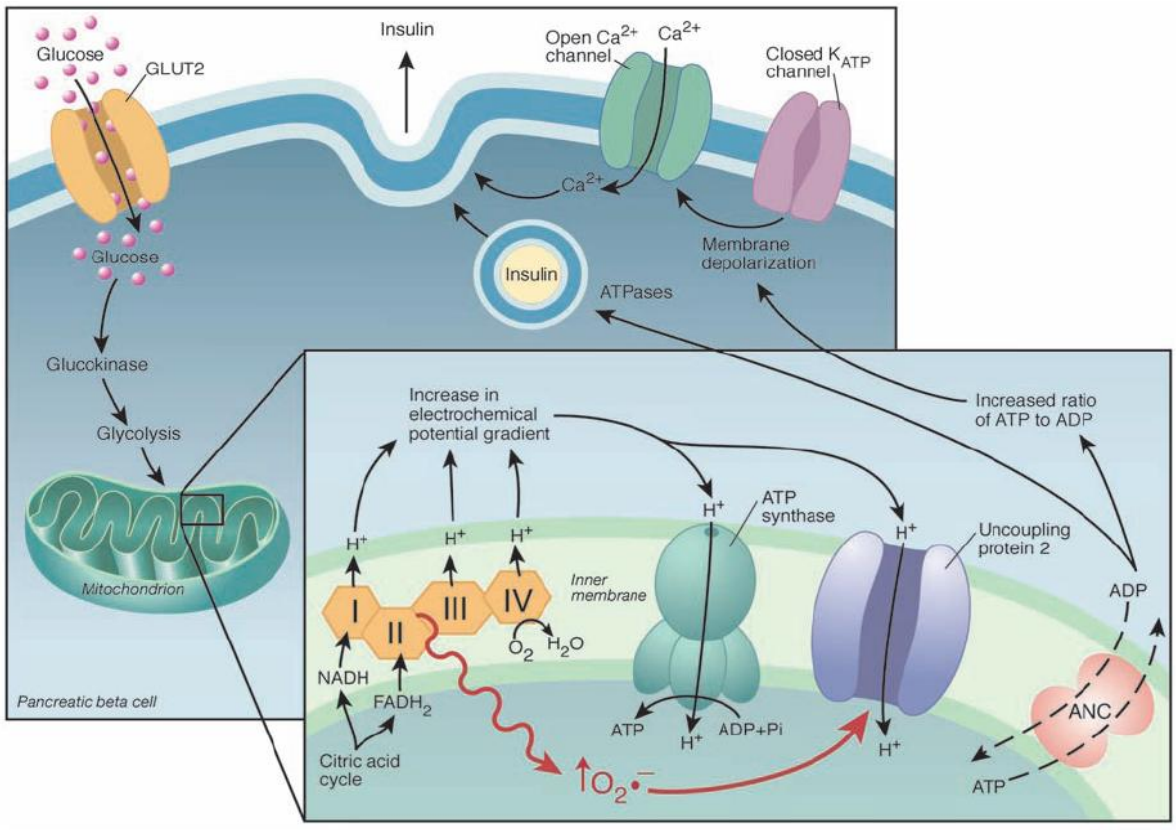
Appendix 1. Chemical structures of EGCG and Rutin



J. Clin. Invest**. 116**: 1802-1812 (2006)

Appendix 2. Mechanisms of β cell failure in Type 2 diabetes

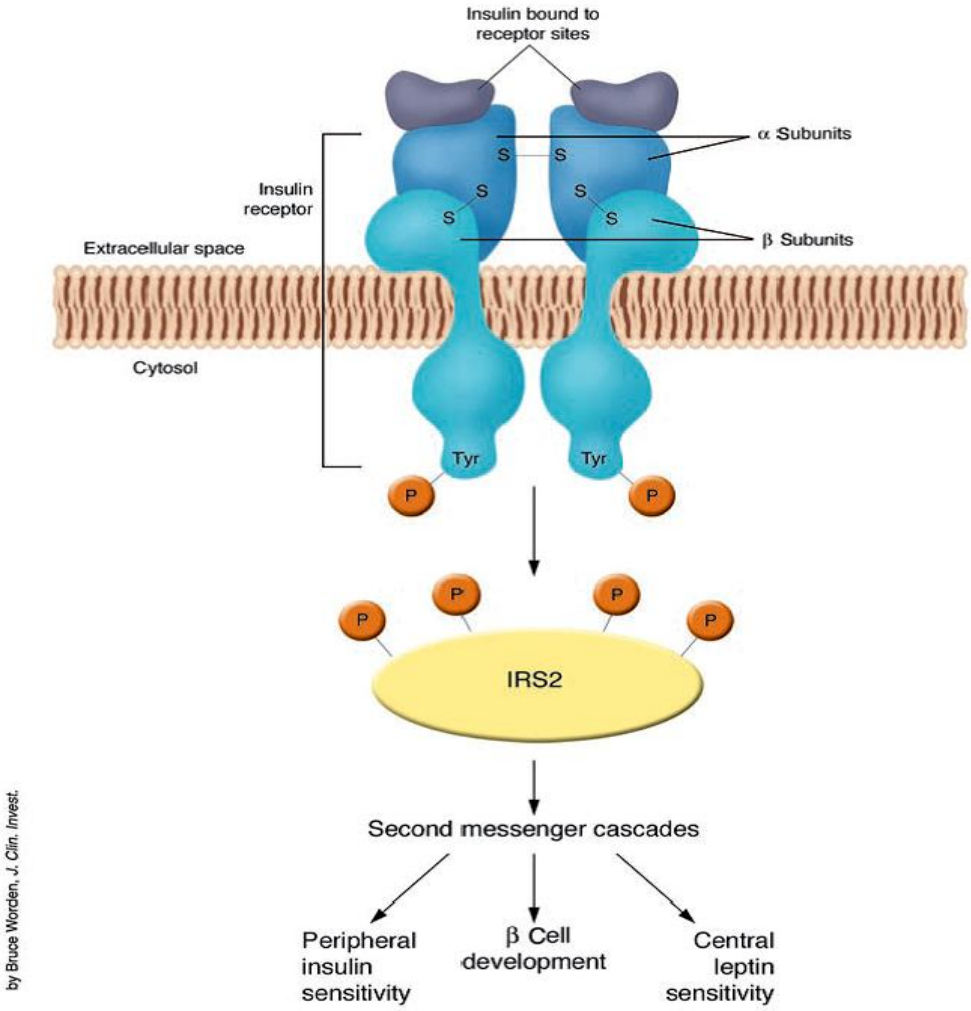
Islet β cell compensation for insulin resistance is sustained provided β cells are robust, resulting in long-term maintenance of NGT. Compensation processes, however, fail if there are genetic or acquired factors that result in susceptible β cells. The defect(s) create weak link(s) in the compensation process that promote β cell dysfunction by mechanisms with initiator roles that result in IGT and early T2D. Hyperglycemia, once established, promotes a further series of mechanisms, under the umbrella of glucotoxicity, that cause severe β cell failure and overt and late T2D. AMPK/Mal-CoA, AMPK/malonyl-CoA signaling network.



Science **307**: 384-387 (2005)

Appendix 3. Glucose-stimulated insulin secretion

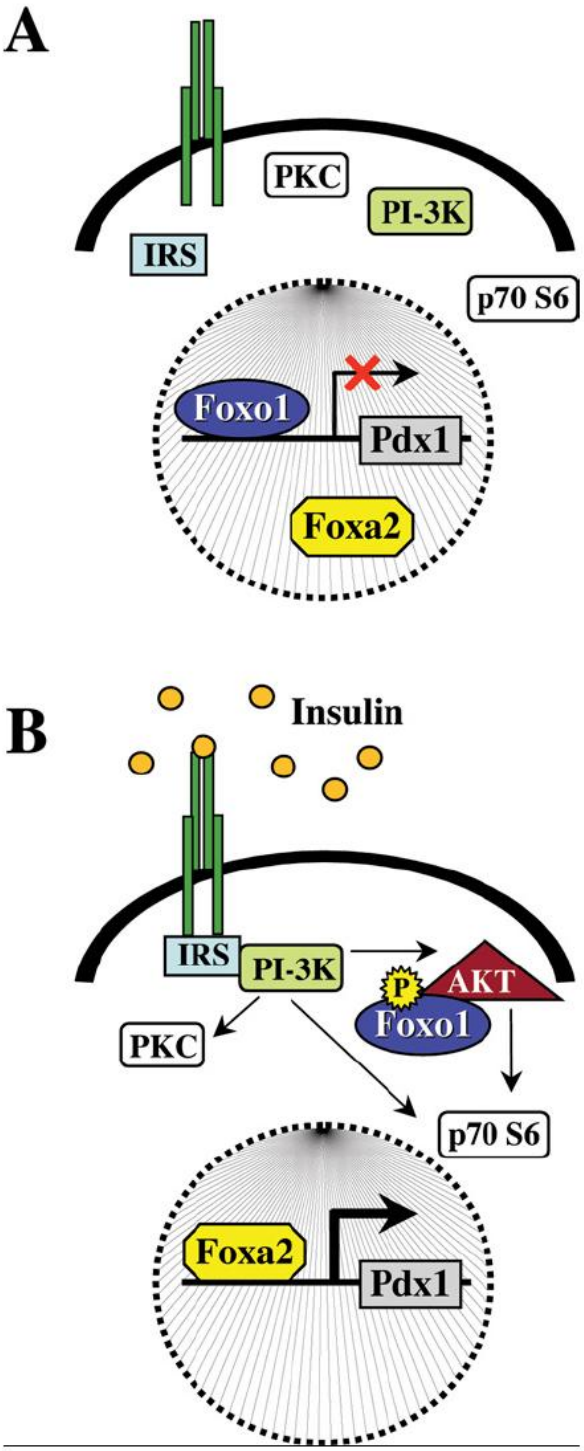
Insulin secretion is coupled to glucose metabolism by the subsequent increase in the ATP/ADP ratio arising from glucose oxidation, which closes K^{ATP} channels. This depolarizes the plasma membrane, opening voltage-gated Ca^{2+} channels with the influx of Ca^{2+} stimulating secretion of insulin.



J. Clin. Invest**. 114**: 886-888 (2004)

Appendix 4. Role of IRS2 in insulin signaling

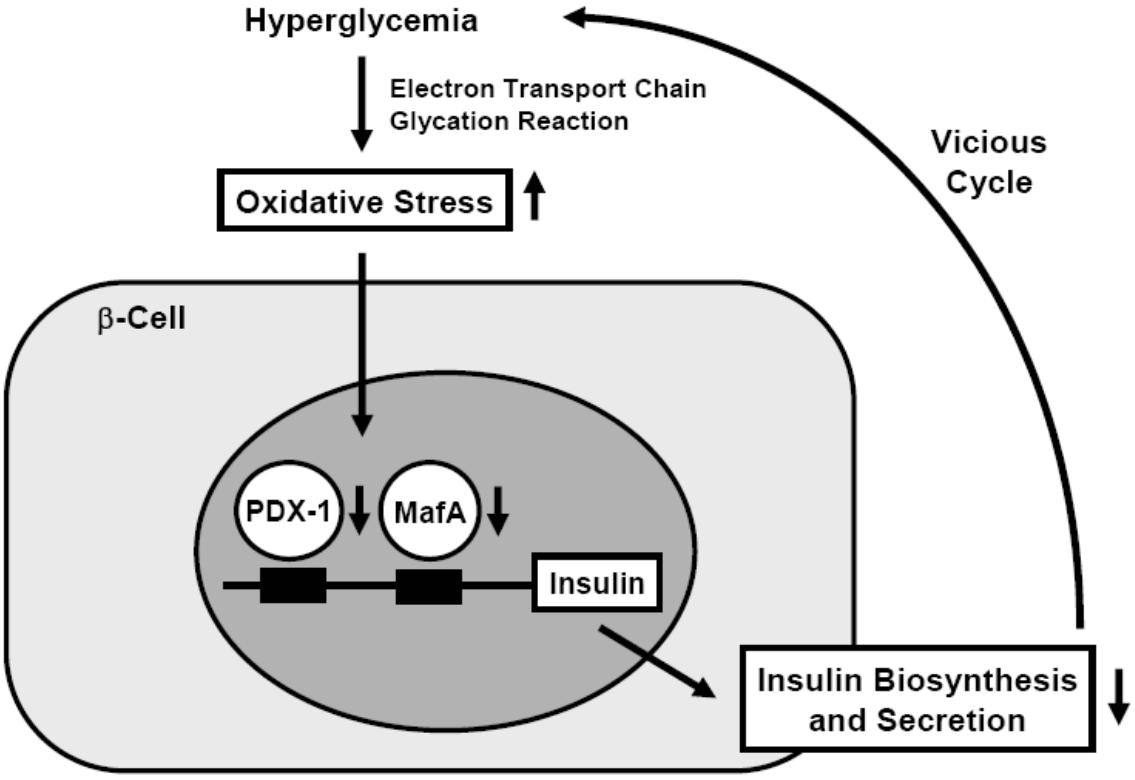
A critical role for IRS2 in the maintenance of peripheral insulin sensitivity, central leptin sensitivity, and proper β cell development in the islets of Langerhans. Thus, IRS2 plays a central role in preserving insulin action in multiple cell types, while reduciton of IRS expression and/or function may be a fundamental cause of the development of insulin resistance, obesity, β cell faliure, and type 2 diabetes.



Appendix 5. Coordinated regulation of Pdx1 by Foxo1 and Foxa2 in the β-cell

(A) In the absence of insulin signalling, Foxo1 binds to Pdx1 and represses transcription. (B) Insulin bound to its receptor stimulates IRS (insulin receptor substrate) proteins to bind to both the insulin receptor and PI-3K (phosphoinositide 3-kinase), resulting in the activation of several proteins. Foxo1 is phosphorylated by Akt and translocates out of the nucleus. Foxa2 then binds to Pdx1 to activate transcription.

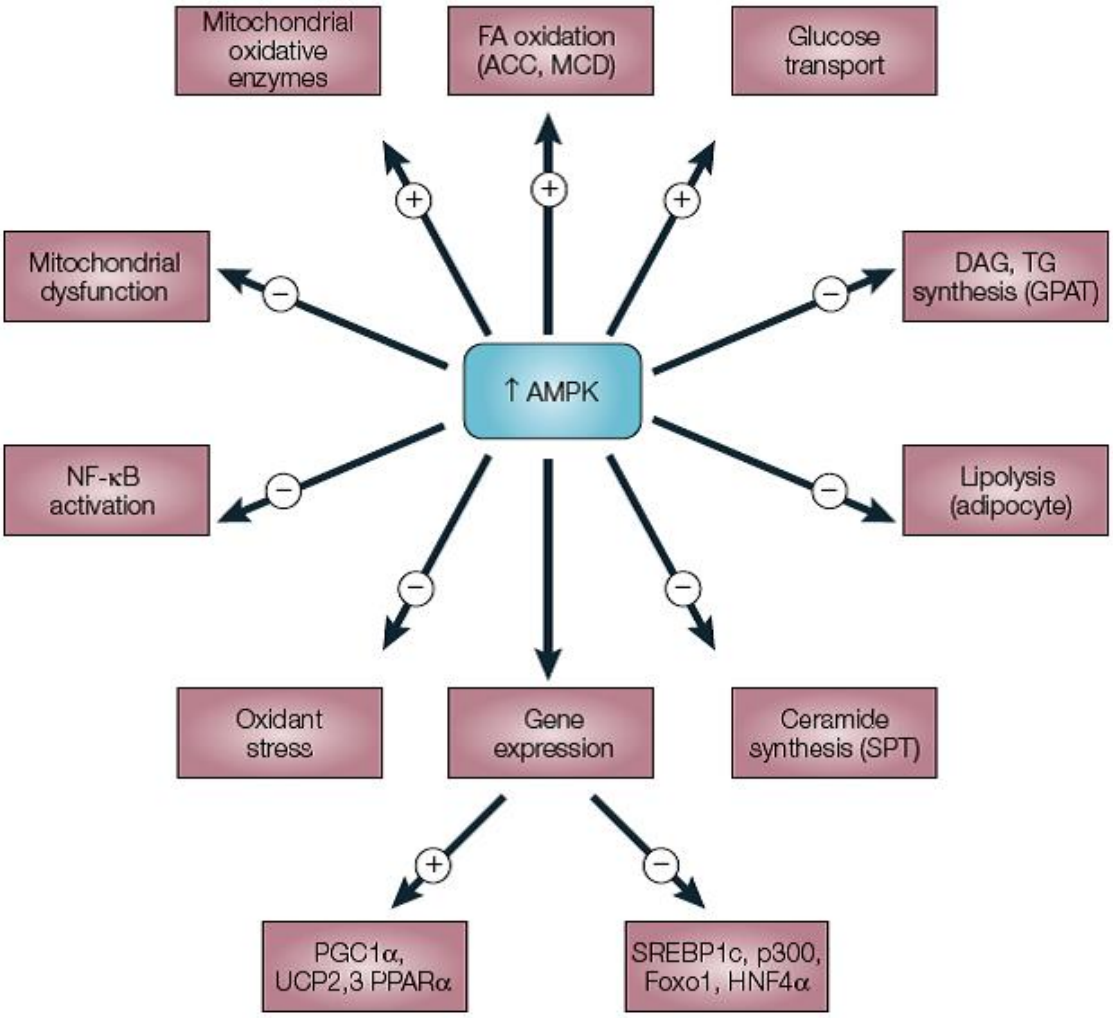
Clinical Science **108**: 195–204 (2005)



Endocr J. **55**(2): 235-52 (2008)

Appendix 6. Role of PDX-1 and MafA in pancreatic β-cell glucose toxicity

Chronic hyperglycemia deterioates β-cell function by provoking oxidative stress, accompanied by reduction of PDX-1 and MafA DNA binding activities. This process is often observed under diabetic conditions and is called β-cell glucose toxicity.



Nat Rev Drug Discov **3**:340-351 (2004)

Appendix 7. Effects of AMPK activation on events that could account for its ability to diminish lipid accumulation, cell dysfunction and insulin resistance