行政院國家科學委員會補助專題研究計畫成果報告

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※　分化後之 3T3-L1 脂肪細胞中胰島素抗拒症表達遺傳學之研究　※

※Expression genetics of insulin resistance syndrome in differentiated 3T3-L1 adipocytes※

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計畫類別：■個別型計畫　　□整合型計畫

計畫編號：NSC **89-2314-B-002-070-**

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一、計畫中文摘要：請於五百字內就本計畫要點作一概述，並依本計畫性質自訂關鍵詞。

關鍵詞：非胰島素依賴型糖尿病，胰島素抗拒症，脂肪細胞，訊息核醣核酸，基因表達

非胰島素依賴型糖尿病在台灣及全球均為主要之新陳代謝疾病之一. 其慢性合併症如動脈硬化,視網膜病變,神經病變,及腎病變,其後果皆頗為嚴重. 因此非胰島素依賴型糖尿病對我們的社會有極大之衝擊. 現在一般認為非胰島素依賴型糖尿病之致病過程大致分為兩方面:即胰島素抗拒症及胰島乙型細胞功能喪失. 在過去十年來,遺傳學之方法已導致發現幾個基因如MODY2 及粒線體基因,其突變可造成胰島乙型細胞無法分泌胰島素.不過這些基因突變約佔非胰島素依賴型糖尿病病人中之不到百分之二. 相形之下,吾等現今對胰島素抗拒症之遺傳機制之了解更顯欠缺.

在此計劃中，我們提出利用一些 3T3-L1 細胞之胰島素抗拒症模式來評估有差異性表現之基因之生物學上的相關性,並進一步界定其功能.我們已逐步建立胰島素抗拒症之細胞培養模式,如加上高量胰島素,TNFα處理等等.胰島素抗拒症之狀態可以葡萄糖代謝;脂解作用加以監測. 我們將萃取其 RNA，再以即時 PCR 及 Microarry 加以監測 mRNA 之差異性表現，接著以 Northern blot 加以證實. 如一基因被發現在胰島素敏感與抗拒之狀態間有差異性表現, 則可用 rosiglitazone 來測試可否調節其表現. 通過這些相關性測試之基因則將被選擇做進一步之分析.其 sense 或 anti-sense 之 cDNA 將被植入 expression plasmids 中,並永續導入 3T3-L1 細胞中,接著做條件式之表現以評估其對胰島素敏感性及其他相關性狀之影響.吾等已將兩個基因植入 expression plasmids，將進行一些功能性研究，這些有差異性表現之基因將來亦可被用於檢驗非胰島素依賴型糖尿病病人之脂肪組織之基因表現.

總而言之,吾等意圖利用一些細胞培養之胰島素抗拒症模式,來探討在脂肪細胞胰島素抗拒症模式中有差異性表現之基因的生物學上之意義.此基礎研究之結果將很容易可找到其臨床應用之相關性.

二、計畫英文摘要：請於五百字內就本計畫要點作一概述，並依本計畫性質自訂關鍵詞。

Keywords：non-insulin dependent diabetes mellitus, insulin resistance syndrome, adipocyte, mRNA, gene expression

Non-insulin dependent diabetes mellitus (NIDDM) is one of the major metabolic disorders both in Taiwan and worldwide. Its chronic complications including atherosclerosis, retinopathy, neuropathy and nephropathy are devastating. Therefore, NIDDM has an enormous impact on our society. The pathogenesis of NIDDM is now generally agreed upon to include two arms, i.e. insulin resistance and islet β cell failure. In the last decade, the classical genetic approach has led to the identification of a few genes, such as MODY2 and mitochondrial tRNA gene, which mutations cause the failure of islet β cells to secret insulin. These mutations make up only less than 2% of NIDDM patients. In contrast, our current understanding of the genetic mechanism of insulin resistance is very deficient.

We have proposed to study the biological relevance of the differentially expressed genes in several insulin resistance models in cell culture. We have set up the models of insulin resistance induced by the treatment of high-dose insulin, TNFα and so on in differentiated mouse 3T3-L1 cells. The status of insulin sensitivity will be monitored by of glucose consumption and lipolysis. The differential expression of genes in the pathways of glucose and lipid metabolism will be monitored with real-time PCR and cDNA microarray followed by the confirmation with Northern blots. If a gene were differentially expressed between insulin sensitive and resistant states, rosiglitazone will be added to investigate whether its expression pattern could be modified. Genes that pass through these relevance tests will be chosen for further study. The conditional expression plasmids containing sense or anti-sense cDNA will be constructed and stably transfected into 3T3-L1 cells to evaluate their effects on insulin sensitivity and the other related phenotypes. We have so far cloned two genes in expression plasmids. Functional assays of these genes on insulin resistance will be soon studied. The genes differentially expressed in these models can also be chosen to screen adipose tissue gene expression in NIDDM patients in the future.

In conclusion, we intend to utilize the cell culture models of insulin resistance to pursue the biological significance of the differentially expressed genes between insulin sensitive and resistant state of adipocytes. The results of this basic study can be easily related to clinical application.

三、結果：

We have set up several models of insulin resistance induced by the treatment of high-dose insulin, TNFα and so on in differentiated mouse 3T3-L1 cells. The main issue during the process of setting up these models was the inconsistency of 3T3-L1 differentiation. We found that 3T3-L1 cells after prolonged propagation may not be optimal for differentiation induction. On the other hand, cells only moderately (around 30%) differentiated may still be successfully used for a second attempt. We have tried several conditions of stimulation on differentiated 3T3-L1 and assay their steady-state consumption of glucose and glycerol release (Fig 1). In differentiated 3T3-L1 adipocytes, we found that higher glucose itself could stimulate glucose consumption. Insulin could stimulate further glucose consumption, however the effect was quite modest. Interestingly, insulin could enhance lipolysis consistent with a recent report (1). In low glucose concentration (100 mg/dL), the effect of glucosamine and dexamethasone on glucose consumption and lipolysis was not obvious. In our pilot experiments with high glucose (450 mg/dL), glucosamine had a significant effect on reducing glucose consumption, whereas dexamethasone had a significant effect on enhancing lipolysis (data not shown). TNFα increased both glucose consumption and lipolysis. We have collected the lysates for these samples. RNA extraction will be performed and real-time PCR, microarray and Northern-blot will be conducted to detect differential gene expression.

We have also cloned human adiponectin and PPAR genes into some expression vectors. We will assay the effects of over-expression, under-expression and expression of mutant genes on the metabolism and gene expression in differentiated 3T3-L1.

The progress of this project has been slow. However, some significant results will be available in the second year of this project.

References:

1. Botion LM, Green A 1999 Long-term regulation of lipolysis and hormone-sensitive lipase by insulin and glucose. Diabetes 48:1691-7.

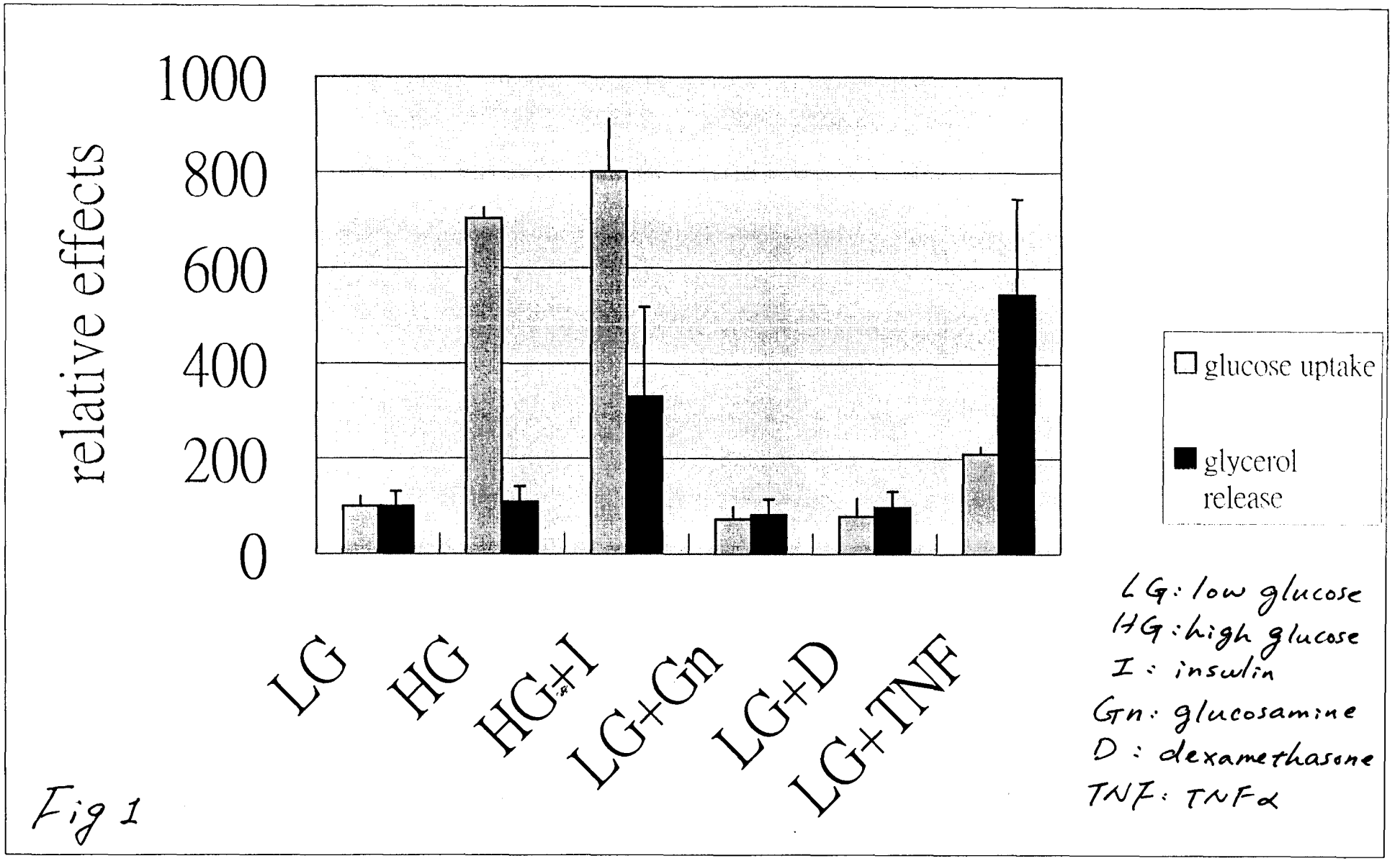
relative effects
1000
800
600
400
200
0
LG
HG
HG+I
LG+Gn
LG+D
LG+TNF
glucose uptake
glycerol release
LG: low glucose
HG: high glucose
I: insulin
Gn: glucosamine
D: dexamethasone
TNF: TNFα

Fig 1