Constitutive and inducible regulation of *PEPCK* isoform genes in human cells

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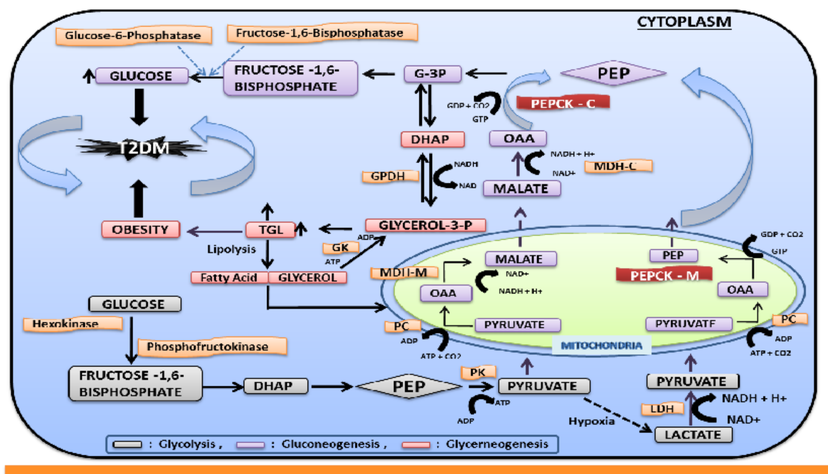
INTRODUCTION

Phosphoenolpyruvate Carboxykinase (PEPCK) catalyzes conversion of GTP dependent oxaloacetate to phosphoenolpyruvate, which is the key rate determining reaction of gluconeogenesis pathway. PEPCK exist in cytosolic (PEPCK-C) and mitochondrial (PEPCK-M) isoforms. Genes coding for PEPCK isoforms are regulated by hormones, steroids, drugs and such others leading to increased glucose production from non-carbohydrate sources. PEPCK isoforms maintain glucose/lipid homeostasis and is being explored as a therapeutic target for treating metabolic diseases. We tested the influence of a naturally occurring compound Genistein, (a soy derived isoflavone) to constitutively regulate PEPCK isoforms to address its potential use as a therapeutic molecule.

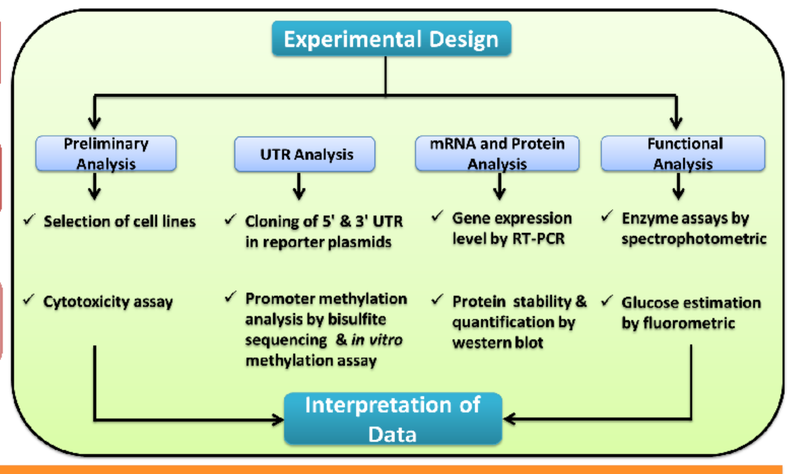
KEY PATHWAYS OF GLUCOSE METABOLISM



OBJECTIVES

1. To investigate the relative expression and function of PEPCK isoforms in human cells
2. To identify genetic/epigenetic factors for controlling PEPCK gene expression
3. To study effect of genistein on PEPCK expression and function

METHODOLOGY



RESULTS & DISCUSSION

Fibroblasts possess decreased expression and function of PEPCK isoforms

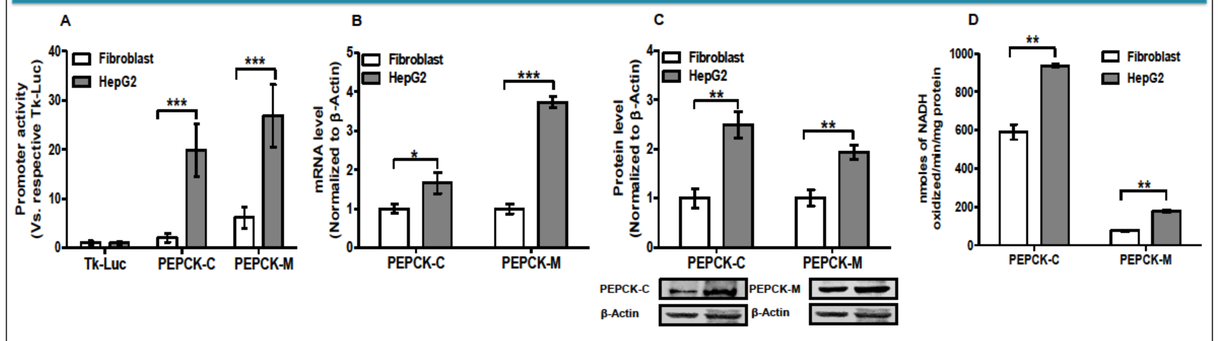


Figure 1: Relative expression and function of PEPCK isoforms in fibroblasts and HepG2 cells.
A) Promoter activity, B) Gene expression, C) Protein quantification, D) Specific activity

Glucose is required for genistein mediated effects for regulation of PEPCK isoform genes

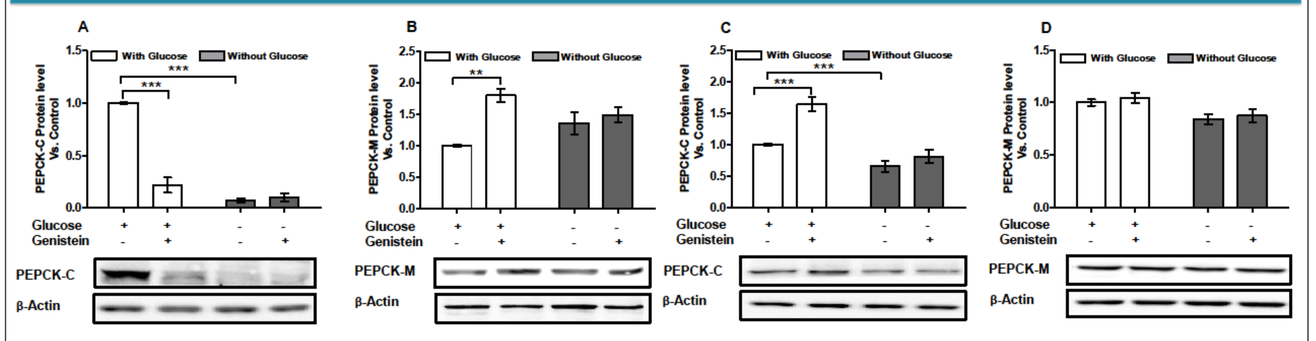


Figure 2: Effect of genistein on expression of PEPCK isoform genes in cells cultured in presence or absence of glucose. A)HepG2 cells PEPCK-C, B) HepG2 cells PEPCK-M, C) Fibroblast PEPCK-C, D) Fibroblast PEPCK-M

Genistein exerts differential effects in genes regulating PEPCK isoforms

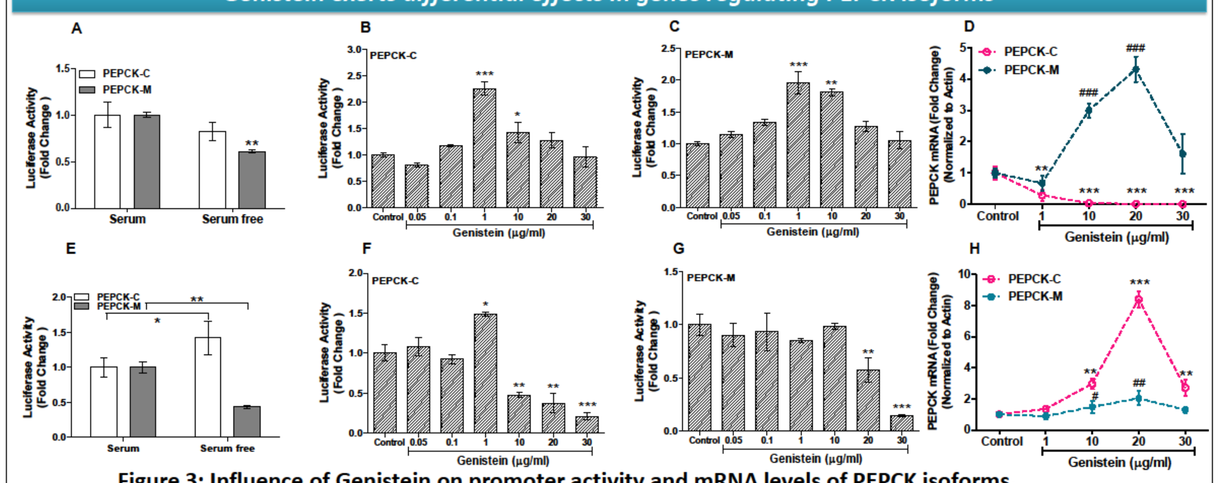


Figure 3: Influence of Genistein on promoter activity and mRNA levels of PEPCK isoforms.
A-C)Promoter activity in HepG2 cells, D)Gene expression in HepG2 cells, E-G)Promoter Activity in Fibroblast cells, H)Gene expression in Fibroblast cells

Genistein induces degradation of PEPCK-C protein and conversely elevated expression of PEPCK-M

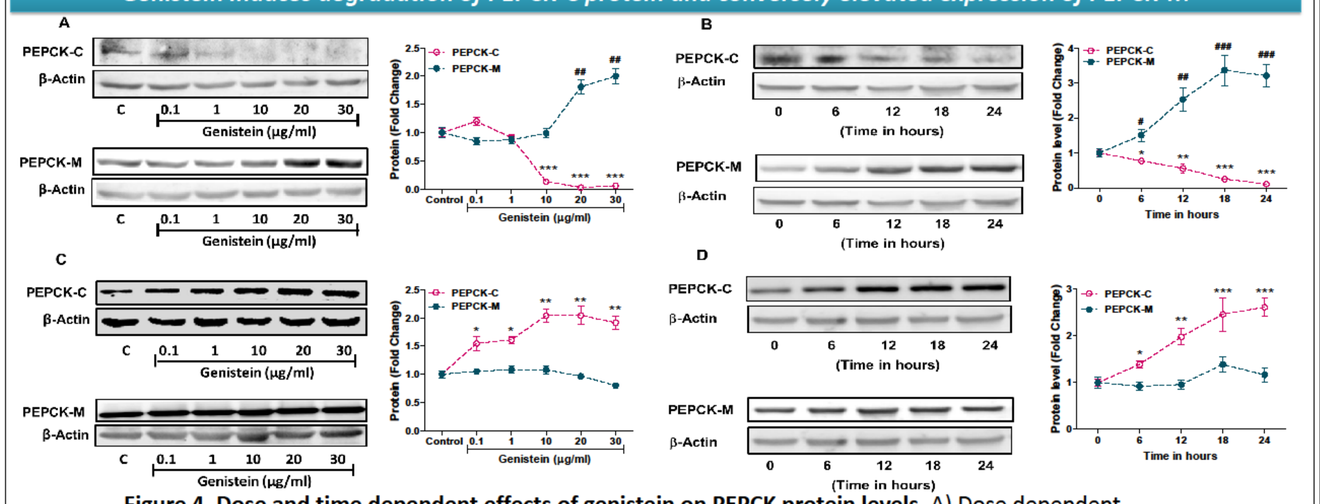


Figure 4. Dose and time dependent effects of genistein on PEPCK protein levels. A) Dose dependent in HepG2,B) Time dependent in HepG2,C)Dose dependent in Fibroblast ,D)Time dependent in Fibroblast

Genistein modulates PEPCK function

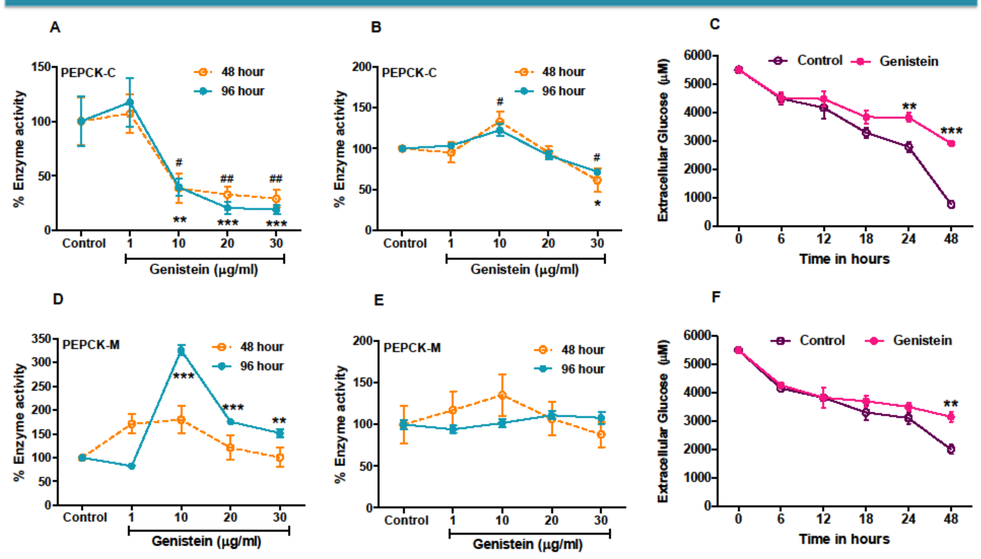


Figure 5: Influence of genistein on PEPCK enzyme activity and glucose release.
A&B) Specific activity C) Glucose estimation in condition media of HepG2 cells
D&E) Specific activity F)Glucose estimation in condition media of Fibroblast cells

Statistical analysis

All experiment data are presented as the mean ± standard error of the mean (SEM). Data were analyzed using the Student's t-test and one-way analysis of variance (ANOVA) .A P value of < 0.05 was considered as statistically significant.

CONCLUSION

We show an evidence for the regulation of PEPCK-C gene by promoter DNA methylation in human fibroblasts, which might be responsible for maintaining baseline PEPCK activity in non-gluconeogenic tissues. We show selective effect of soy isoflavone genistein on PEPCK isoform genes in hepatic and extra hepatic cell types by increasing the expression of PEPCK-M as a compensatory mechanism and there by maintaining glucose homeostasis in cells as a pro-survival effect.

Genistein mediated regulation of PEPCK isoform genes is post-transcriptional

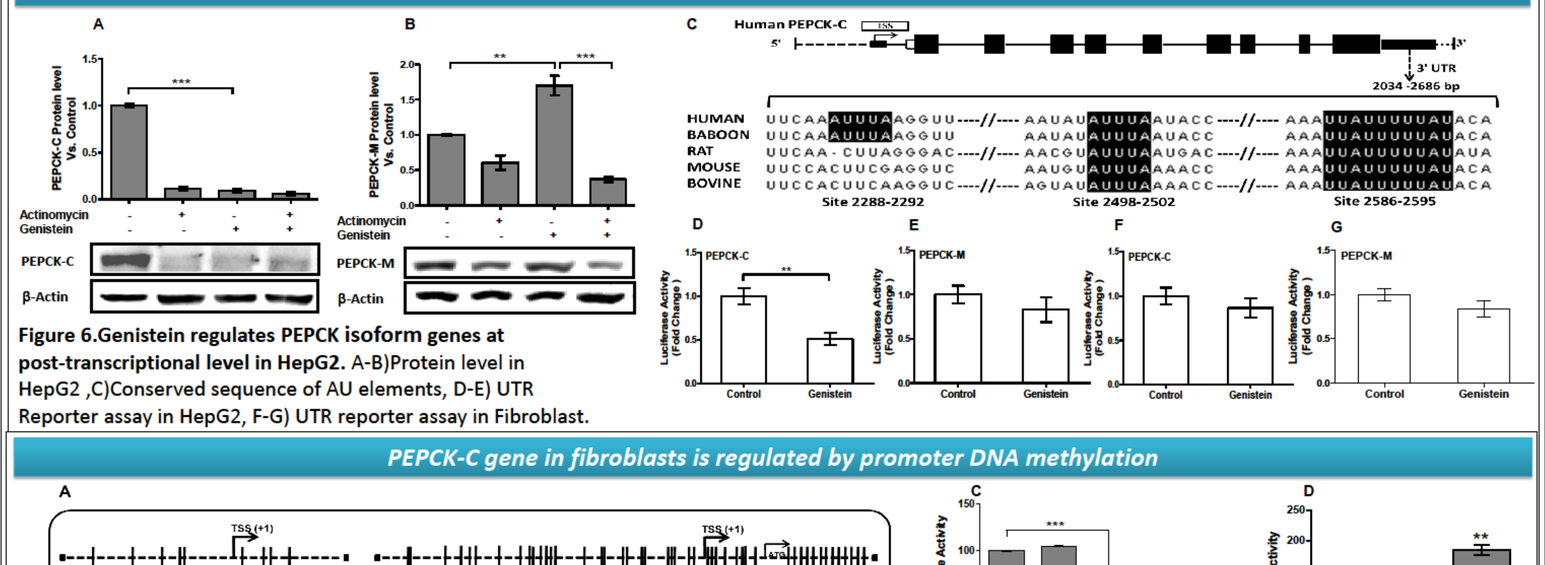


Figure 6.Genistein regulates PEPCK isoform genes at post-transcriptional level in HepG2. A-B)Protein level in HepG2 ,C)Conserved sequence of AU elements, D-E) UTR Reporter assay in HepG2, F-G) UTR reporter assay in Fibroblast.

PEPCK-C gene in fibroblasts is regulated by promoter DNA methylation

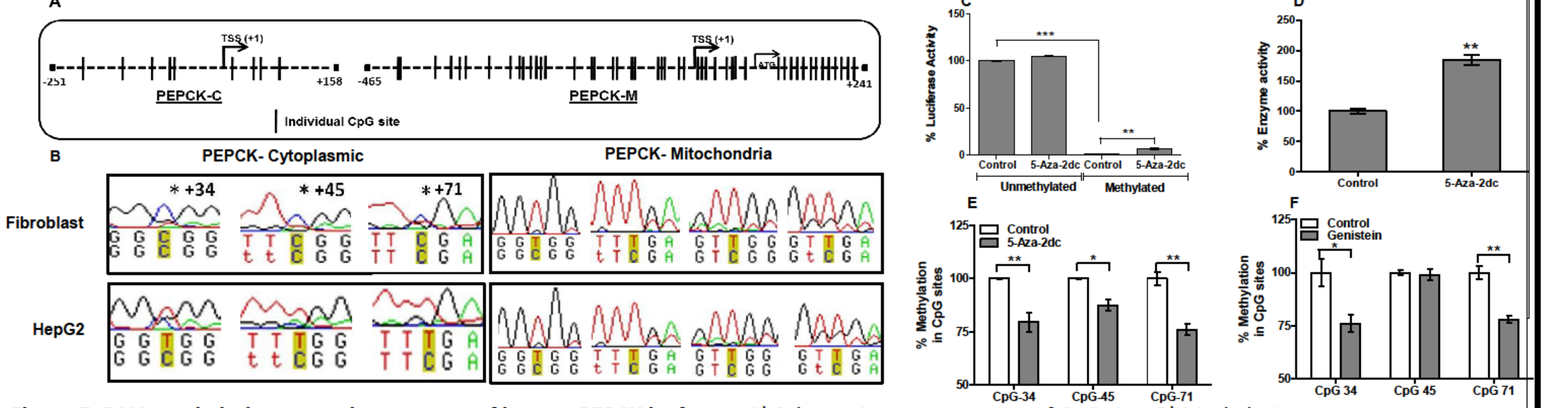


Figure 7: DNA methylation status in promoter of human PEPCK isoforms. A) Schematic representation of CpG sites,B) Methylation status of CpG sites,C)Artificial methylation reporter assay in HepG2,D)Specific activity in Fibroblast,E&F)Bisulfite sequencing

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Reproduction, endocrine disruptors and signalling

venu seenappa

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