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Graded and Pathway-Specific Integration of Insulin, Glucagon, and Time Shapes Hepatic Transcriptional Programs

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Abstract

Introduction: Insulin and glucagon are principal regulators of hepatic metabolism [1,2], yet their transcriptional effects are often examined using binary perturbation models that obscure graded and context-dependent regulation. Most studies contrast hormone presence versus absence, limiting resolution of dose-dependent and multivariate integration. This study aimed to determine how continuous variation in insulin and glucagon concentrations, together with incubation time, independently shapes hepatic transcriptional pathway activity.

Methodology: Primary mouse hepatocytes were exposed to multiple concentrations of insulin and glucagon across different incubation times. RNA sequencing was performed to quantify transcriptomes. Pathway activity was inferred using Gene Set Variation Analysis (GSVA) across all 50 Hallmark gene sets [3-4]. For each pathway, additive linear models were fitted to estimate independent contributions of log2-transformed insulin concentration, log2-transformed glucagon concentration, and incubation time. Effect sizes and adjusted R^2 values were used to assess pathway-specific regulation and relative contributions of hormonal and temporal effects.

Results & Discussion: Insulin emerged as the dominant hormonal regulator, exerting graded and pathway-specific effects rather than binary responses. Metabolic and anabolic pathways, including glycolysis, mTORC1 signaling, and cholesterol homeostasis, showed positive insulin-associated coefficients with variable effect sizes, indicating differential sensitivity to insulin concentration. In contrast, glucagon rarely exhibited strong independent main effects. When present, glucagon-associated coefficients were typically opposite in direction to insulin, consistent with antagonistic rather than synergistic regulation.

Only a limited subset of pathways-primarily central metabolic signaling hubs-showed strong contributions from both hormones, and these effects were predominantly antagonistic. No pathway demonstrated strong concordant regulation by insulin and glucagon. Incubation time

was a major independent determinant of pathway activity. Cell cycle-and transcription-associated programs, including E2F, MYC, and G2M checkpoint pathways, were strongly time-dependent and frequently exceeded hormonal contributions, indicating progressive transcriptional adaptation during in vitro incubation.

Conclusion: Hepatic transcriptional pathway regulation reflects graded, pathway-specific insulin responsiveness, limited independent glucagon effects, and strong time-dependent adaptation. These findings demonstrate that endocrine integration at the pathway level cannot be fully captured by binary hormone perturbation designs and underscore the importance of continuous multivariate modeling approaches [5].

Keywords: Insulin, glucagon, hepatocytes, pathway analysis, GSVA

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