Hexosamines Provoke Membrane Cholesterol Accrual, Filamentous Actin Loss, and GLUT4 Dysregulation in Adipocytes through Transcriptional Activation of Specificity Protein 1

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Abstract

The hexosamine biosynthesis pathway (HBP) serves as a sensor of excess nutrient bioavailability and has been implicated in the pathogenesis of type 2 diabetes. Previous study observed that hyperinsulinemic culturing conditions akin to those seen clinically activate the HBP provoking gains in plasma membrane (PM) cholesterol content in L6 myotubes and 3T3-L1 adipocytes. This, in turn, compromised the cortical filamentous actin (F-actin) structure necessary for the proper incorporation of the insulin sensitive glucose transporter GLUT4 into the membrane. The mechanism(s), however, by which HBP activation provokes PM cholesterol accrual, remains unclear. Here, the hypothesis that HBP engages a cholesterolgenic transcriptional response resulting in PM cholesterol accrual/toxicity was tested. In 3T3-L1 adipocytes, pathophysiologically relevant doses of hyperinsulinemia (0.25, 0.5, and 5 nM) resulted in a dose-dependent gain in PM cholesterol as well as mRNA and protein levels of HMG-CoA reductase (HMGR), the rate limiting enzyme in cholesterol synthesis. Immunoprecipitation experiments demonstrated that hyperinsulinemia induced elevations in *O*-linked N-acetylglucosamine post-translational modification of the cholesterolgenic transcription factor specificity protein 1 (Sp1). This modification was prevented in cells in which the HBP was inhibited. Chromatin immunoprecipitation demonstrated that hyperinsulinemia induced a ~4 fold increase in the affinity of Sp1 to the promoter region of HMGR, which was lost with HBP inhibition. Luciferase assays confirmed that this altered binding resulted in a ~50% increase in promoter activity of this cholesterolgenic gene. Hyperinsulinemia also augmented Sp1 binding to the promoter of the sterol response element binding protein gene, resulting in increased total and nuclear content of this factor. To further delineate the role of Sp1 in this process, a specific inhibitor, mithramycin (MTR), of Sp1 binding to DNA was employed. This inhibitor prevented against hyperinsulinemia-induced gains in HMGR and PM cholesterol as well as F-actin loss. Importantly, this treatment corrected the impaired insulin-stimulated GLUT4 translocation and glucose transport induced by hyperinsulinemia. These data suggest hyperinsulinemia-induced HBP activity provokes cholesterol synthesis and PM cholesterol accrual/F-actin loss that compromises GLUT4/glucose transport regulation by insulin.