Glucose uptake in isolated cardiomyocytes.

Glucose transport assays were performed in triplicate in 12-well (22 mm diameter) laminin-coated tissue culture plates. Laminin-plated isolated cardiomyocytes were washed twice with 1 ml of glucose-free DMEM. Then 1 ml of glucose-free DMEM (37°C) containing 0–1 nM insulin or 0–100 nM IGF-1, 1 mM pyruvate, and 0.1% BSA was added. After 40 minutes, 10 µl of a 2-deoxyglucose mix containing 130 µl of glucose-free DMEM, 15 µl of a 100-mM 2-deoxyglucose solution, and 5 µl of a 1- µCi/ µl ^{3}H 2-deoxyglucose (NEN Life Science Products Inc., Boston, Massachusetts, USA) was added. After 30 minutes, the medium was aspirated and the cells washed twice with 1 ml of cold PBS. Cells were lysed in 500 µl of NaOH 1N for 20 minutes at 37°C. A 40- µl aliquot of the lysed cells was used for measuring protein content solution using a Micro BCA Protein Assay Kit (Pierce Chemical Co., Rockford, Illinois, USA). A 400- µl aliquot of lysed cells was counted to determine the specific activity of ^{3}H 2-deoxyglucose.