Metformin (Figure 1) is an anti-diabetic drug widely used to treat type 2 diabetes mellitus.

Studies show that in vivo, metformin decreases hepatic glucose production and intestinal glucose transport, and increases intestinal glucose utilization, all of which contribute to blood glucose-lowering effects of metformin in diabetes. Increased intestinal glucose utilization is thought to contribute to metformin-induced intestinal lactic acidosis. Studies show that the highest metformin accumulation occurs in the intestine following oral administration, which raises the question whether high metformin intestinal concentrations modulate cell function. Since intestinal glucose absorption occurs from the apical (AP) and basolateral (BL) membranes, it warrants investigating whether high metformin concentrations affect intestinal glucose absorption and glucose transporter (GLUT) translocation to membranes of intestinal cells.

Little is known about how metformin regulates GLUT transporters, although some evidence shows that metformin promotes their translocation to the AP membrane. Under normal physiology, GLUT2 is known to translocate to the apical membrane of intestinal epithelia in response to a meal. A previous study suggests an increase in GLUT2 translocation to the AP membrane of mouse jejunal tissue following metformin treatment due to the activation of the cellular energy sensor, AMP-activated protein kinase (Figure 2).

This study aims to elucidate the effect of metformin on glucose uptake and transport via GLUT transporter translocation to AP and BL membranes in Caco-2 cell monolayers, a well-established model of human intestinal epithelia.

**Purpose**

**Methods**

Caco-2 Cell Culture. Caco-2 cells were cultured at 37°C in Eagle's minimum essential medium supplemented with 10% fetal bovine serum, 1% nonessential amino acids, and 1% antibiotic-antimycotic in 5% CO₂ and 90% relative humidity. Cells were seeded at 120,000 cells/cm² on 12-well Transwell™ plates and cultured for 21-28 days prior to transport experiments.

Real-Time (RT) PCR. Total RNA was isolated from Caco-2 cells and reverse transcribed using a Superscript® III First-strand synthesis kit (Invitrogen). Human small intestinal total RNA was purchased from Zyagen. RT-PCR was conducted using Taqman assays (Applied Biosystems) to determine relative expression of GLUT1-3. All mRNA levels were normalized to 18s rRNA.

2-deoxy-D-Glucose (2DG) Uptake. Caco-2 cell monolayers were preincubated for 30 min with transport buffer (Hank's Balanced Salt Solution with 10 mM HEPES) with or without 5 mM metformin. To initiate uptake, 2DG solution (10 mM) was added to the donor compartment. After 10 minutes, cells were washed with ice cold transport buffer three times and then lysed with 0.1 N NaOH/0.1% SDS. 2DG was quantified by liquid scintillation spectrometry.

2-deoxy-D-Glucose (2DG) Transport. Caco-2 cell monolayers were preincubated for 30 min with transport buffer with or without 10 mM metformin. To initiate transport, 2DG solution (100 mM) was added to the AP compartment. At the designated timepoints, 10 μM aliquots were collected from the BL compartment, and 2DG was quantified by liquid scintillation spectrometry.

**Results**

Metformin (10mM) treatment was associated with a trend towards decreased AP uptake of 2DG and its increased BL uptake.

**Conclusions**

- Trends favoring decreased AP glucose uptake may be reflective of the inhibitory effect of metformin on energy-dependent transport of 2DG concentrations not high enough to recruit GLUT 2 transporters.
- Trends towards increased BL 2DG uptake and increased AP to BL overall transport may be indicative of a mechanism to increase glucose utilization.
- Lack of statistical significance may be due to experimental conditions of high 2DG concentration that saturated transport.
- Further studies are needed to confirm the effects of metformin using lower concentrations of 2DG.

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**References**