Genetic Knockout of Intestinal Hexokinase Domain Containing Protein-1 Affects Enterocyte Glucose Transport in Mice Fed High Fat Diet

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ABSTRACT

Hexokinase domain containing protein-1, or HKDC1, is a widely expressed novel hexokinase that is genetically associated with elevated 2-hour gestational blood glucose levels during an oral glucose tolerance test, suggesting a role for HKDC1 in postprandial glucose regulation during pregnancy. Our earlier studies utilizing transgenic mice containing whole-body *HKDC1* knockdown, or mice in which hepatic HKDC1 was overexpressed or knocked out, indicated that HKDC1 is important for whole-body glucose homeostasis in aging and pregnancy, through modulation of glucose tolerance, peripheral tissue glucose utilization, and hepatic energy storage. However, our knowledge of the precise mechanisms by which HKDC1 regulates postprandial glucose homeostasis under normal and diabetic conditions is lacking. As the intestine is the main entry portal for dietary glucose, and since HKDC1 is highly expressed within the intestine, in this study we assessed and characterized the *in vivo* significance of intestine-specific HKDC1 in regulating glucose homeostasis under normal and obesogenic conditions. We developed an intestine-specific HKDC1 knockout mouse model, HKDC1$^{Int-/-}$, utilizing Cre-mediated recombination of *HKDC1* in which Cre was expressed under the control of the *villin* gene promoter, leading to genetic knockout of *HKDC1* solely within the intestinal epithelium. Mice were maintained until 28 weeks of age on either a normal chow diet or a high fat diet to develop obesity, hyperglycemia, and insulin resistance. While no overt glycemic phenotype was observed, 28-week-old HKDC1$^{Int-/-}$ mice fed a high fat diet exhibited an increased glucose excursion following an oral glucose load compared to mice expressing intestinal HKDC1. This finding was not due to differences in insulin levels, whole-body insulin tolerance, or gluconeogenesis, nor was it a result of alterations in enterocyte glucose utilization or a reduction in peripheral skeletal muscle glucose uptake. Furthermore, the enhanced glucose excursion was related to transport of glucose through the intestinal epithelium, as mice administered an intraperitoneal glucose load did not exhibit alterations in post-load glycemic excursion. Assessment of intestinal glucose transporters in high fat diet-fed HKDC1$^{Int-/-}$ mice indicated an increased expression of GLUT2 in the enterocyte apical membrane in the fasting state. Taken together, our results indicate that intestine-specific HKDC1 contributes to postprandial glycemic regulation by modulating dietary glucose transport across the intestinal epithelium under conditions of enhanced metabolic stress, such as obesity, hyperglycemia, and diabetes.

BACKGROUND

Postprandial Hyperglycemia

- A risk factor for cardiovascular mortality in diabetics and contributes to adverse outcomes in pregnancy
- Therapeutics targeting intestinal glucose entry are limited

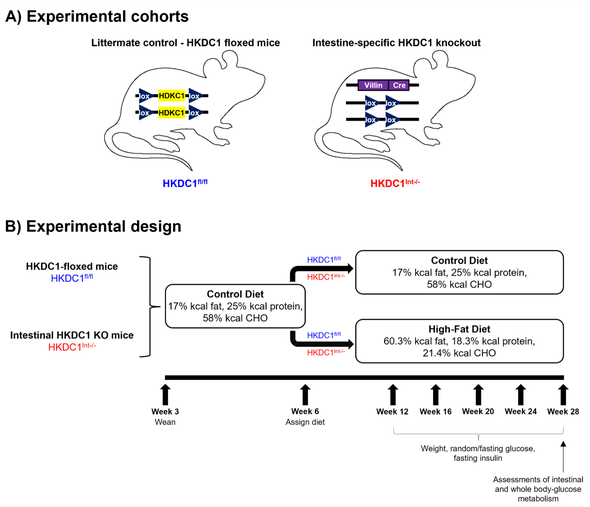
Hexokinase Domain Containing Protein-1 (HKDC1)

- A novel hexokinase associated with 2-hour gestational blood glucose levels during an oral glucose tolerance test
- Whole-body knockdown of *HKDC1* leads to (1) increased glucose excursion following an oral glucose load, (2) reduced peripheral glucose uptake, and (3) reduced hepatic energy storage at day 15 of mouse pregnancy and in non-pregnant 28-week-old mice
- The above studies suggest a link between HKDC1 and postprandial glycemic control

Purpose of this Work

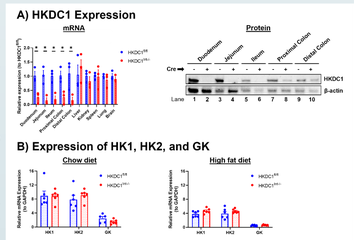
- HKDC1 is highly expressed within the intestinal epithelium, but its precise function within the intestine is not well understood
- The purpose of this work is to elucidate the role of intestine-specific HKDC1 in dietary glucose uptake under both normal and obesogenic/diabetic conditions

EXPERIMENTAL DESIGN



RESULTS

Figure 1: Knockout of intestinal *HKDC1* does not affect *HKDC1* expression in other tissues or intestinal hexokinase mRNA expression. HKDC1 mRNA expression was assessed in intestinal and non-intestinal tissues using quantitative PCR and the expression of *HKDC1* in HKDC1$^{Int-/-}$ mice relative to littermate controls is shown **(A, *left panel*)**. To correlate mRNA findings with HKDC1 protein expression, intestinal mucosa were scraped, homogenized, centrifuged, and a sample of supernatant analyzed with SDS-PAGE and immunoblotting **(A, *right panel*)**. Assessment of hexokinase mRNA levels in intestinal epithelium in the presence or absence of intestinal HKDC1 was assessed with qPCR **(B)**. *-P < 0.05



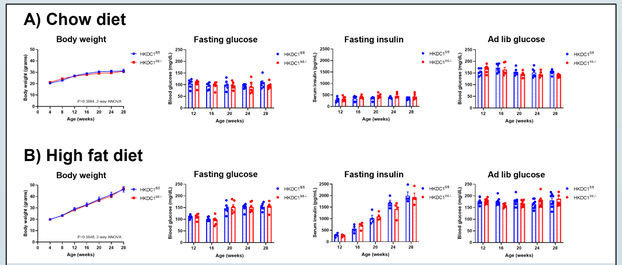


Figure 2: Knockout of intestinal *HKDC1* does not result in an overt glycemic phenotype. Chow- **(A)** and high fat diet-fed **(B)** mice were analyzed at four-week intervals until 28 weeks of age. Fasting blood glucose and serum insulin measurements were conducted after an overnight 16-hour fast.

RESULTS

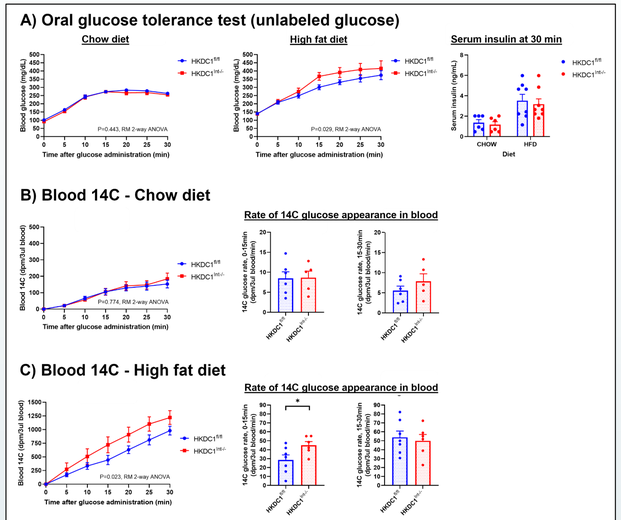


Figure 3: HKDC1$^{Int-/-}$ mice chronically fed a high fat diet demonstrate an increased blood glucose excursion following an oral glucose load. Following a 16-hour overnight fast, 28-week-old mice fed a chow or high fat diet were administered a 2 g/kg body weight oral bolus of glucose **(A)** or an oral glucose bolus supplemented with 2-[1-^{14}C]-deoxy-O-glucose (14C glucose) **(B and C)**. Blood measurements were made at five-minute intervals until 30 minutes. Measurements of radiolabeled glucose are reported as disintegrations per minute (dpm) per 3 µL of blood. Rates of 14C glucose excursion between 0-15 min and 15-30 min are shown. Additionally, blood was collected in heparinized capillary tubes at 0 and 30 minutes, centrifuged, and insulin level was measured in the serum using an ELISA. *-P < 0.05

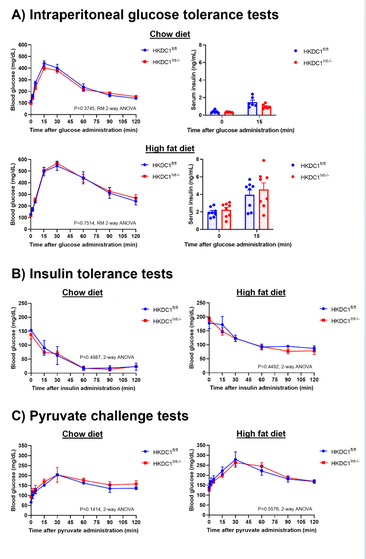


Figure 4: Compared to littermate controls, HKDC1$^{Int-/-}$ mice exhibit similar glucose excursion after an intraperitoneal glucose load, whole body insulin sensitivity, and gluconeogenesis. After a 16-hour overnight fast, 28-week-old mice were given 2 g/kg body weight glucose via intraperitoneal injection, bypassing the intestinal epithelium **(A)**. Blood glucose levels were assessed at multiple time points over the first two hours, and serum insulin measurements were assessed at 0 and 15 minutes using an ELISA. To assess insulin sensitivity and gluconeogenesis, mice were fasted for 6 hours prior to administration of 0.75 units per kg Humalog insulin **(B)**, and fasted overnight prior to administration of 2 mg/g body weight sodium pyruvate **(C)**, respectively. Blood glucose levels were assessed at various time points for two hours.

RESULTS

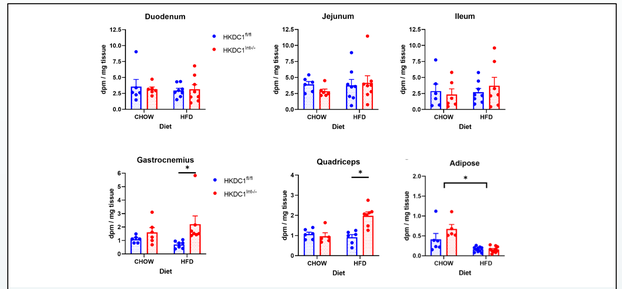


Figure 5: Compared to littermate controls, HKDC1$^{Int-/-}$ mice chronically fed a high fat diet exhibit similar intestinal and greater skeletal muscle glucose utilization. 30 minutes into a radiolabeled oral glucose tolerance test, sections of small intestine **(top row),** skeletal muscle and perigonadal adipose tissue **(bottom row)** were homogenized, centrifuged, and supernatants were placed onto anion-exchange columns. Columns were eluted and the amount of phosphorylated (trapped) 2-[1-^{14}C]-deoxy-O-glucose was measured using a scintillation counter. *-P < 0.05

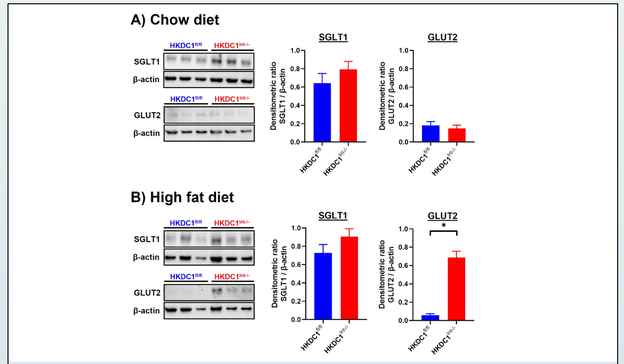


Figure 6: HKDC1$^{Int-/-}$ mice chronically fed a high fat diet exhibit increased enterocyte apical surface GLUT2 after an overnight fast compared to littermate controls. Following an overnight fast, 28-week-old mice fed normal chow **(A)** or high fat diet **(B)** were euthanized, duodenal and jejunal mucosa collected, and the enterocyte apical membrane was isolated and purified through centrifugation using magnesium chloride to aggregate and precipitate non-brush border structures. Immunoblotting for apical SGLT1 and GLUT2 was performed. Densitometric analyses were performed using NIH ImageJ software. *-P < 0.05

CONCLUSIONS & FUTURE DIRECTIONS

- **Intestine-specific HKDC1 is an important modulator of postprandial glucose absorption and asserts its greatest effect under conditions of higher metabolic stress, such as obesity**
- **Intestine-specific HKDC1 modulates enterocyte apical GLUT2 expression in the fasting state of mice fed a high fat diet**
- **Knockout of intestine-specific HKDC1 in mice fed a high fat diet leads to increased skeletal muscle glucose uptake**
- **Examination of glucose transporter dynamics during an OGTT, and skeletal muscle glucose uptake processes, are critical to elucidating the precise mechanism(s) by which intestinal HKDC1 modulates postprandial glycemic control**