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TITLE PAGE

Title

Disordered regulation of intestinal glucose sensor, transporters and absorption in critically ill humans and mice

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AMD was jointly responsible for study conception and design, obtaining funding, acquisition of data, interpretation of data in humans and statistical analysis, and drafting and submission of manuscript.

RLY was jointly responsible for study conception and design, obtaining funding, acquisition of data, supervision of all RNA extraction and quantitative PCR measurements in humans and mice, interpretation of data in mice and statistical analysis, and drafting and submission of manuscript.

CKR was responsible for study design, performance of endoscopies, analysis and interpretation of data, obtaining funding, drafting of the manuscript and critical revision of the manuscript for important intellectual content.

AK was responsible for subject enrolment and safety of endoscopies performed in critically ill patients.

NQN was responsible for performance of endoscopies and critical revision of the manuscript for important intellectual content.

NC, ZM and **BC** were responsible for studies performed in mice and RNA extraction and quantitative PCR measurements in humans and mice.

MJS, **JAS** and **TvB** were responsible for data collection and storage.

MJC was responsible for obtaining funding and critical revision of the manuscript for important intellectual content.

MH was responsible for postdoctoral supervision of AMD and RLY, obtaining funding, drafting of the manuscript and critical revision of the manuscript for important intellectual content.

ABSTRACT

Objective

Providing effective enteral nutrition is important during critical illness. In health, glucose is absorbed from the small intestine via sodium-dependent glucose transporter-1 (SGLT-1) and glucose transporter-2 (GLUT2), which may both be regulated by intestinal sweet taste receptors (STR). We evaluated the effect of critical illness on glucose absorption and expression of intestinal SGLT-1, GLUT2 and STR in humans and mice.

Design

Prospective observational study in humans and mice

Setting

Intensive Care Unit and University-affiliated research laboratory

Subjects

Human subjects were 12 critically ill patients and 12 healthy controls. In the laboratory sixteen-week old mice were studied.

Intervention

Human subjects underwent endoscopy. Glucose (30g) and 3-O-methylglucose (3-OMG; 3g), used to estimate glucose absorption, were infused intraduodenally over 30min. Duodenal mucosa was biopsied before and after infusion. Mice were randomized to cecal ligation and puncture (CLP) to model critical illness (n = 16), or sham laparotomy (control) (n = 8). At day 5, mice received glucose (100mg) and 3-OMG (10mg) infused intraduodenally prior to mucosal tissue collection.

Measurements

Quantitative PCR was performed to measure absolute (human) and relative levels of SGLT-1, GLUT2 and STR (T1R2) transcripts. Blood samples were assayed for 3-OMG to estimate glucose absorption.

Main Results

Glucose absorption was threefold lower in critically ill humans than controls (P=0.002) and reduced by a similar proportion in CLP mice (P=0.004). In critically ill patients, duodenal levels of SGLT-1, GLUT2 and T1R2 transcript were reduced 49% (P<0.001), 50% (P=0.009) and 85% (P=0.007), while in the jejunum of CLP mice SGLT-1, GLUT2 and T1R2 transcripts were reduced by 55% (P<0.001), 50% (P=0.002) and 69% (P=0.004).

Conclusions

Critical illness is characterized by markedly diminished glucose absorption, associated with reduced intestinal expression of glucose transporters (SGLT-1 and GLUT2) and STRs transcripts. These changes are paralleled in CLP mice.

INTRODUCTION

The importance of enteral nutrition during critical illness is well recognized (1). However, the provision of adequate nutrition via this route is frequently compromised because absorption is impaired (2). This can result in the presence of undigested nutrient in the large intestine, which increases the risk of diarrhea, gas formation and consequent abdominal distension, all of which occur frequently in the critically ill, and may have adverse effects (3).

The effect of critical illness on carbohydrate absorption is of particular interest given carbohydrate is the predominant macronutrient in standard enteral formulae and is an important determinant of glycaemia (4, 5). 3-O-methylglucose (3-OMG) is a glucose analog that is absorbed via the same transporters but is not metabolized (6). Measuring postprandial serum concentrations of 3-OMG provides an estimate of glucose absorption in animals, healthy humans and the critically ill (4, 7-9). In health, glucose is primarily absorbed from the intestinal lumen to the enterocyte via the sodium-dependent glucose co-transporter (SGLT-1), which is located in the brush border membrane, and potentially, via the facilitative glucose transporter-2 (GLUT2), which may relocate to the apical membrane (4).

Studies in animals have established that intestinal levels of SGLT-1 and GLUT2 transcript and protein are increased in the presence of a broad array of sweet ligands, via an intestinal sweet taste receptor pathway (10-13). The lingual detection of sugars and sweeteners is dependent on activation of a G-protein coupled sweet taste receptor (STR), a heterodimer of T1R2 and T1R3 subunits (T1R2/R3) (14). Sweet taste molecules are also present in the proximal small intestine of humans, and transcript levels, which are likely to reflect subsequent protein levels, are responsive to systemic factors such as glycaemia (15). If intestinal STR mechanisms function in humans as in animals, it is likely that these

receptors link the presence of luminal glucose to the increased expression and availability of SGLT-1 and GLUT2 for glucose transport (10-12).

Hyperglycemia occurs frequently in the critically ill, is usually unrelated to antecedent diabetes, and affects outcomes adversely (16, 17). While the pathophysiology of this hyperglycemia is poorly defined, inadequate insulin and excessive glucagon secretion relative to blood glucose concentrations are considered key contributing factors (16, 17). In health, the gastrointestinal tract is the principal determinant of insulin secretion in response to ingested glucose, via the release of the 'incretin' hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (13). GLP-1 is present in intestinal L-cells, which express STRs, and these have been linked functionally to secretion of GLP-1 in both animals and humans (13, 14, 18). Accordingly, if critical illness is associated with a reduction in intestinal STR levels this is likely to impact adversely on the secretion of GLP-1 and, thereby, glycemic control.

The use of an animal model of illness facilitates the study of novel therapies. Cecal ligation and puncture (CLP) mice are a well-described model of severe sepsis (19). CLP mice can be studied in the 'recovery' phase of their illness, which is comparable to the period in which clinicians would like to optimize nutritional therapy in the critically ill.

The primary objective of this study was to determine whether intestinal transcript levels of SGLT-1, GLUT2 and STR (T1R2) are reduced in human critical illness. Secondary objectives were to evaluate the effect of critical illness on glucose-induced secretion of GLP-1, GIP, insulin and glucagon in humans and to establish an animal model that replicates the molecular and functional intestinal abnormalities observed in critically ill humans.

MATERIALS AND METHODS

Critically ill patients and healthy volunteers

Mechanically-ventilated critically ill patients who were suitable to receive enteral nutrition via a small intestinal feeding catheter were studied between September 2010 and August 2011. Patients were excluded if they were aged less than 18 years, pregnant, known to have diabetes, had a contraindication to endoscopy, had a history of surgery on the stomach, duodenum, or small intestine, were receiving drugs known to alter platelet aggregation or thrombus formation, had an International Normalized Ratio (INR) > 1.5 or platelet count < 50,000.

Healthy volunteers were matched as closely as possible to the critically ill subjects for age, sex and body mass index, and the same exclusion criteria applied.

Protocol

Patients were studied after a fast of at least 8 hours. If insulin was being administered, infusions were ceased at least 2 hours before the study. Healthy subjects ingested a liquid nutrient meal the night before the study to replicate recent exposure to enteral feeding in the critically ill (20). Subjects were studied approximately at the same time of day (0900h) with blood sampled from an intravenous cannula in health and an existing intra-arterial cannula in patients. A small diameter (5.3 mm) video endoscope was inserted via the nose or mouth into the second part of the duodenum using minimal insufflation of air (15). Three mucosal biopsies were collected and stored in RNAlater (Qiagen) and the endoscope was left in situ. From 0 to 30 minutes (T0 - T30) liquid nutrient (30 g glucose + 3 g 3-OMG (Sigma-Aldrich) dissolved in water to a total volume of 150 ml) was infused directly into the duodenum over 30 minutes (i.e. 4 kcal/min). At T30, three further duodenal biopsies were taken, after which the endoscope was removed. Blood was sampled at T0, T10, T20 and T30 for blood glucose and 3-OMG concentrations.

Mouse model of critical illness - Cecal ligation and puncture (CLP)

Twenty-seven 16 week-old male C57 mice were randomized to cecal ligation and puncture (CLP) or sham laparotomy (control) groups. The CLP model has been previously detailed by other investigators (19). In the sham group the caecum was located, but neither ligated nor punctured. Mice received intensive support for 5 days following surgery with twice-daily fluid resuscitation (1 ml subcutaneous saline) and Terramycin (Pfizer, 1mg/100g body weight) and Butorphanol (Pfizer, 0.5g/100g) for the first 2 days. Mice were monitored twice daily for survival and standardized clinical disease scores, which were summed for the entire experiment (Cumulative Disease Index) (21). On day 5 all surviving mice (n = 24) were fasted for 2 h then anaesthetized with isoflurane (Ceva Delvet), a laparotomy performed and the proximal duodenum cannulated. A warmed liquid nutrient (100mg glucose bolus +10mg of 3-OMG in 1ml of water) was infused into the proximal duodenum over 30 min. Mice were humanely killed by pentobarbitone (Troy Laboratories) overdose, with blood from terminal bleeds collected for assessment of 3-OMG concentrations. Small intestinal samples were collected into RNAlater (Qiagen) for molecular assays.

Data analysis

Quantification of gene expression by real time RT-PCR

RNA was extracted from human and mouse tissues using RNeasy Mini or Micro kits (Qiagen) following manufacturer instructions, with RNA yield and quality determined by NanoDrop (NanoDrop Technologies, USA). Quantitative real time reverse transcriptase PCR (RT-PCR) was used to determine absolute levels of T1R2, SGLT-1 and GLUT2 transcript in 25 ng of total human RNA, and transcript levels relative to ß-actin in mice. Primers for these targets were used as validated primer assays (QuantiTect, Qiagen; Supplementary table 1) or in the case of absolute PCR standards, designed from target sequences obtained from the NCBI nucleotide database (Supplementary table 2)

(15). Minimum detectable levels for human SGLT-1 and T1R2 transcripts were 2 x 10^3 copies; when levels were not detected, this threshold value was substituted.

RT-PCR was performed on a Chromo4 (MJ Research) real time instrument attached to a PTC-200 Peltierthermal cycler (MJ Research) using a QuantiTect SYBR Green one-step RT-PCR kit (Qiagen) according to the manufacturer’s specifications. Cycling conditions were reverse transcription at 50ºC for 30 min, 95ºC for 15min, then 45 cycles PCR at 94ºC for 15s, followed by 55ºC for 30s and 72ºC for 30s. A melt curve was generated (60-95ºC) to verify the specificity and identity of the RT-PCR products; product size was confirmed on an electrophoresis gel (Bio-Rad Laboratories). Each assay was performed in triplicate and included internal no-template and no-RT controls.

Blood glucose, hormone and 3OMG assays

Blood glucose was measured at the bedside using a portable glucometer (Medisense Optimum, USA). Both serum and plasma were separated by centrifugation (3200rpm for 15mins at 4°C). Samples were then stored at -70°C until assayed. Serum concentrations of 3-OMG were measured in both humans and mice using liquid chromatography/mass spectroscopy with an assay sensitivity of 0.0103 mmol/L (8). Serum insulin was measured in humans by enzyme-linked immunosorbent assay (ELISA) (Mercodia, Sweden) with sensitivity 1.0 mU/L, and coefficient of variation 3.1% within and 5.9% between assays.

Blood samples for glycated hemoglobin (HbA1c), glucagon, and incretin hormones were collected into chilled 5-mL ethylenediaminetetraacetic acid tubes. HbA1c was measured using cation exchange high performance liquid chromatography. Plasma glucagon was measured by radioimmunoassay (Millipore, USA); the minimum detectable limit was 20 pg/mL and intra- and inter-assay coefficients of variation were 8.5% and 22.5% respectively. Total GLP-1 was measured by

ELIZA (Epitope Diagnostics) with sensitivity 0.05 pmol/L, and intra- and inter-assay coefficients of variation 5.4% and 3.9%. Plasma GIP was measured by radioimmunoassay (22). The minimum detectable limit was 2pmol/L, with intra- and inter-assay coefficients of variation 3.2% and 9.3% respectively.

Immunohistochemistry

Fixed tissues were cryoprotected (30% sucrose in phosphate-buffered saline), embedded in cryomolds then frozen. Frozen tissues were sectioned at 6-10μm (Cryocut 1800, Leica Biosystems, Germany) and thaw-mounted onto gelatin-coated slides. Immunoreactivity in human tissues was detected using a rabbit T1R2 primary (H90, 1:400, SC-50305, Santa Cruz Biotechnology), a monoclonal GLUT2 (1:800, AB85715, Abcam) and GIP primary (1:800, AB30679, Abcam) and goat SGLT-1 (1:400, SC-47398, Santa Cruz) and GLP-1 primary antibodies (C-17, 1:400, SC-7782, Santa Cruz). All were visualised using species-specific secondary antibodies conjugated to Alexa Fluor dyes (1:200 in PBST) according to earlier methods (15, 23). Antigen retrieval (S1700, Dako) was performed for T1R2 and SGLT-1 according to manufacturer instructions. Nucleated epithelial cells immunopositive for the individual targets were counted per square millimeter of high power field and averaged over a minimum of 10 intact transverse sections per subject.

Protocols were approved by the Human Research Ethics Committee of the Royal Adelaide Hospital and the Animal Ethics Committee of the Institute of Medical and Veterinary Science, respectively. Informed consent for each patient was obtained from the next of kin and volunteers gave written consent.

Statistical analysis

The primary endpoints were intestinal transcript copy numbers or relative expression of SGLT-1, T1R2 and GLUT2. The sample size was based on our previous data comparing duodenal RNA levels in ambulant patients with type 2 diabetes, healthy volunteers or mice (15, 23). Twelve subjects and a minimum of 8 mice per group gave 80% power to detect a difference in RNA expression between groups of 30%, accepting an α error of 0.05. Mice were randomised 2:1 into CLP and sham groups based on best practice mortality of 50% in this model (24); a higher survival rate was achieved in this study, allowing additional CLP mice to reach study endpoints. Data are reported as median (range). Areas under curves (AUC_{0-30}) were calculated using the trapezoidal rule. Mann-Whitney Tests were used to determine differences between patient and healthy groups, and CLP and control mice. Wilcoxon Signed Rank Tests were used to determine significance within group variables. Linear relationships were evaluated using Spearman's Rank Test. Statistical analyses were performed using SPSS version 17.0. All P values are two sided.

RESULTS

Study in critically ill patients

Twelve patients and twelve healthy subjects were studied; none had an abnormal macroscopic appearance of the small intestinal mucosa. The two groups were well matched (Table 1).

Duodenal transcript levels

SGLT-1 was the most abundant transcript in the duodenum of healthy subjects, with 30% lower levels of GLUT2 ($P < 0.001$) and much lower levels of T1R2 ($P < 0.001$, Figure 1A).

Fasting levels of SGLT-1 transcript were 49% lower in critically ill patients compared to healthy subjects ($P < 0.001$, Figure 2A) while fasting levels of GLUT2 were 50% lower ($P = 0.009$, Figure 2B). T1R2 transcript levels were 85% lower in fasted critically ill patients ($P = 0.007$, Figure

2C) and were undetectable in 4 patients at baseline and one patient after glucose infusion. Acute glucose infusion did not significantly alter transcript levels of SGLT-1, GLUT2 or T1R2 in either patients or healthy subjects (i.e. T0 and T30 levels were similar; P > 0.10 for patients and healthy subjects).

Glucose absorption and glycemia

Fasting and post-infusion blood glucose concentrations were similar in critically ill patients and healthy controls (at T0: critically ill: 6.0 (5.0 - 9.7) vs. healthy: 5.7 (4.9 - 6.8) mmol/L; P = 0.18, and at T30: 8.3 (6.7 - 12.7) vs. 9.3 (5.8 - 11.6) mmol/L; P = 0.35). Serum 3-OMG concentrations were, however, 3-fold lower in critically ill patients (P = 0.002, Figure 3A).

Insulin and glucagon

Fasting insulin concentrations were similar in patients and healthy subjects (P = 0.55), while after glucose infusion, concentrations tended to be lower in the critically ill (P = 0.052), so that the magnitude of increase in insulin was less in the critically ill (P = 0.017, Table 2). Glucagon concentrations were greater in the critically ill during fasting (P = 0.004), and after glucose infusion (P = 0.010, Table 2).

Glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide

GLP-1 concentrations were greater in the critically ill during fasting (P = 0.004), but not after glucose infusion (P = 0.80), although the increment in GLP-1 did not differ significantly (P =0.35, Table 2). Fasting, glucose-stimulated and incremental GIP concentrations were unaffected by critical illness (P = 0.35, P = 0.016 and P = 0.052 respectively, Table 2).

Relationships between variables

Healthy subjects: Significant relationships were evident between transcript levels of SGLT-1 and GLUT2 at baseline in healthy subjects (rho = 0.71; P = 0.009). There were, however, no other significant relationships between transcript levels, or between transcript levels and plasma levels of blood glucose, 3-OMG, GLP-1 or GIP in healthy subjects at any time point.

Critically ill patients: Relationships were evident between T1R2 copy number at baseline and blood glucose levels at baseline (rho = 0.76; P = 0.004) and at 30 min (rho = 0.77; P = 0.004). SGLT-1 copy number also related to fasting blood glucose levels in these patients (rho = 0.58; P = 0.048). SGLT-1 copy number at baseline was strongly associated with fasting plasma concentrations of GLP-1 (rho = 0.74; P = 0.006) and GIP (rho = 0.74; P = 0.006), and 30 min concentrations of GLP-1 (rho = 0.58; P = 0.047) but not GIP.

Immunohistochemistry

Immunolabelling for T1R2 was evident in single cells dispersed throughout the duodenal epithelium in healthy subjects (Figure 4). These cells showed a homogenous distribution of label throughout the cytoplasm, were largely open or 'flask' shaped and found with equal frequency within villi or crypts. In dual labelling experiments, 19 ± 11% of T1R2 labeled cells co-expressed GLP-1, while 13 ± 8% of L- cells co-expressed T1R2 (Figure 4A). In a similar manner, 15 ± 10% of T1R2 labeled cells co-expressed GIP, while 12 ± 8% of K-cells co-expressed T1R2 (Figure 4B). SGLT-1 labelling was detected in the luminal membrane of duodenal epithelial cells, while GLUT2 was present in basolateral aspects of these cells in healthy subjects (Figure 4C, D).

Mouse model of critical illness

Sixteen of the 19 mice (84%) survived to day 5 following CLP surgery, while all 8 mice survived sham operation to day 5 (Figure 5A). CLP surgery was associated with significant morbidity, based on a cumulative disease index (P = 0.03; Figure 5B).

Jejunal transcript levels

As in humans, SGLT-1 was the most abundant transcript in mice, with 8.3-fold lower levels of GLUT2 (P < 0.001, Figure 1B) and much lower levels of T1R2 in sham mice (P < 0.001). Levels of SGLT-1 transcript were 55% lower in CLP mice compared to sham mice after 5 days (P < 0.001, Figure 2D), while GLUT2 levels were halved (P = 0.002, Figure 2E). T1R2 expression was 69% lower in CLP mice compared to sham mice (P = 0.004, Figure 2F).

Glucose absorption and glycemia

Fasting blood glucose did not differ between CLP and sham operated mice (CLP: 6.0 (2.7 - 9.4) vs. sham: 6.8 (3.5 - 10.9) mmol/L; P = 0.45), nor was there a significant difference in blood glucose concentrations at T30 (CLP: 11.2 (7.4 – 23.2) vs. sham: 16.2 (5.7 – 26.1) mmol/L; P = 0.30). 3-OMG concentrations at T30, however, were 3-fold lower in CLP compared to sham mice (P = 0.004, Figure 3B).

Relationships between variables

Sham-operated mice: There were no significant relationships between T1R2, SGLT-1 or GLUT 2 transcript levels and blood glucose or 3-OMG concentrations. Relationships were evident, however, between transcript levels of T1R2 and SGLT1 (rho = 0.70; P = 0.006), T1R2 and GLUT2 (rho = 0.71; P = 0.006) and SGLT1 and GLUT2 (rho = 0.79; P < 0.001).

CLP mice: There were no relationships between transcript levels and 3-OMG concentrations, however T1R2 levels at baseline negatively associated with blood glucose concentrations at baseline (rho = -0.65; P = 0.043) and at 30 min (rho = -0.66; P = 0.050). This was not seen for SGLT-1 or GLUT2. CLP mice showed a similar positive association between T1R2 and SGLT-1 levels (rho = 0.51; P = 0.024), T1R2 and GLUT2 levels (rho = 0.45; P =0.048) and SGLT1 and GLUT2 (rho = 0.57; P = 0.009) as seen in sham mice.

DISCUSSION

This is the first study to evaluate potential molecular mechanisms underlying disordered nutrient absorption during critical illness. The primary observation is that critical illness is associated with markedly reduced intestinal levels of SGLT-1, GLUT2 and STR (T1R2) transcripts in both humans and a mouse model, providing a plausible explanation for the carbohydrate malabsorption observed during critical illness (4, 10, 20, 25).

The optimal amount of calories that should be administered as carbohydrate in mechanically ventilated critically ill patients remains uncertain. Studies evaluating the effect of energy delivery on clinical outcomes, including mortality, have yielded conflicting results (26, 27). However, providing fewer calories may lead to significant late morbidity with poorer functional outcomes following hospital discharge (28, 29), leading to speculation that provision of ‘full’ feeding may protect against excessive catabolism and improve function in survivors (30). However increasing delivery of nutrients into the small intestine in excess of absorptive capacity is unlikely to be beneficial, and may be deleterious, if undigested carbohydrate passes into the large intestine leading to gas formation, abdominal distension, or diarrhea (20) – all of which are frequently observed in enterally-fed critically ill patients (3). For these reasons, identification of the mechanisms underlying nutrient malabsorption,

and the development of validated small animal models in which to study novel therapies directed at improving absorption, are likely to be important for improving outcomes in the critically ill.

The mechanisms underlying reduced SGLT-1 and GLUT2 expression in the critically ill are uncertain, but are likely to include disease-induced changes in cellular signaling within the intestinal epithelium (31). In animals it is established that the activation of intestinal STR has the capacity to regulate levels and function of SGLT-1, and in rats, the translocation of GLUT2 to the apical membrane (10, 12). We observed a linear relationship between T1R2 expression and expression of both SGLT1 and GLUT2 in mice in the current study, indicating that critical illness-induced reduction in intestinal STR may limit the ability of luminal glucose to upregulate SGLT-1 and GLUT2 effectively. Accordingly, the effect of novel therapies to stimulate STR, such as artificial sweeteners, could potentially increase the availability of SGLT-1 and GLUT2 transporters, and thereby enhance absorptive capacity, without exacerbating glycemic excursions. Such a hypothesis can now be evaluated in the CLP mouse model, with the potential to yield outcomes relevant to critically ill humans.

Our data support previous observations that not only is glucose absorption lower in the critically ill, but subsequent metabolism is disordered (4, 20). Consistent with previous studies, increased glucagon with inadequate insulin secretion appeared important determinants of disordered glucose metabolism in this group (16, 17). Given recent evidence of the essential role of SGLT-1 in incretin hormone release in rodents (32, 33) we hypothesized that GLP-1 and GIP concentrations would be reduced in the critically ill when compared to health. In contrast, concentrations of these incretin hormones were at least maintained in patients, and fasting GLP-1 concentrations were increased. A possible explanation for the latter observation is that as a result of impaired nutrient absorption undigested carbohydrate and lipid or excessive bile salts remain in the distal small intestine

and colon even after an 8 hour fast, leading to continuing stimulation of GLP-1 secretion from L-cells in these regions (4, 34, 35).

A particular strength of this study is that the same molecular and functional abnormalities that occurred within the small intestine of critically ill patients were observed in mice following CLP. In addition, the load and rate of glucose administered into the small intestine were controlled by direct intraduodenal infusion; this is important because the rate of nutrient delivery from the stomach into the small intestine is a major determinant of glucose absorption, glycaemia and the secretion of GLP-1 and GIP (36), and gastric emptying is known frequently to be delayed in the critically ill (20, 37).

This study has several limitations. We did not quantify levels of SGLT1, GLUT2 and T1R2 protein in human or mouse mucosa; however, given the marked difference in both transcript and 3-OMG absorption in both species, it is probable that protein levels would be affected similarly. While we demonstrated linear relationships between T1R2 and SGLT-1 expression in mice that were standardized to receive a septic insult and resuscitation, these relationships were not apparent in humans, nor were linear relationships evident between SGLT-1 expression and 3-OMG concentrations in either species. However the sample size was small and the critically ill patient cohort was a heterogeneous group studied at various times during their illness. Accordingly, the study is likely to be underpowered to detect relationships, particularly if such relationships are complex and non-linear. There are other factors, such as flow of chyme within the intestinal lumen and mesenteric blood flow, which influence nutrient absorption, and may be adversely affected by critical illness (4, 9, 20, 38). While luminal and mesenteric blood flows may influence nutrient absorption it is likely that transporter numbers are a pivotal determinant of glucose absorption in the critically ill. Given that the prolonged presence of an endoscope may be uncomfortable for some volunteers, we limited the duration of intraduodenal glucose infusion to 30 minutes. It would now be of interest to examine the

effects of longer periods of glucose-stimulation. Lastly, the patients studied were admitted due to a variety of injuries or illness. This may be relevant as it has been reported that traumatic brain injury stimulates GLUT2 but inhibits SGLT-1 in isolated rat jejunum (39).

In conclusion, critical illness attenuates glucose absorption and markedly reduces the transcript numbers of glucose transporters and sweet taste receptors in the small intestinal mucosa.

REFERENCES

FIGURE LEGENDS

Figure 1. Comparative expression of intestinal SGLT-1, GLUT2 and T1R2 in healthy human and sham laparotomy mouse intestine.

A. Absolute copy number of SGLT-1 [1.9×10^8 ($1.6 - 3.7 \times 10^8$)], GLUT2 [1.5×10^8 ($3.7 \times 10^7 - 3.8 \times 10^8$)] and T1R2 transcript (*inset*) [1.5×10^5 ($4.2 \times 10^4 - 5.6 \times 10^5$)] present in duodenal biopsies from healthy subjects.

B. Expression of SGLT-1 [1.421 (0.683 – 2.303)], GLUT2 [0.151 (0.089 - 0.237)] and T1R2 transcript (*inset*) [0.831 (0.146 – 3.006) x 10^{-4}] relative to β-actin in mouse jejunal scrapings; § $P < 0.001$ when compared to SGLT-1

Figure 2. Critical illness-induced changes in expression of intestinal SGLT-1, GLUT2 and T1R2 in patients and resuscitated mice.

Absolute copy number of **A.** SGLT-1 [at T0: critically ill: 9.5×10^7 copies ($7.3 \times 10^7 - 2.0 \times 10^8$) vs. healthy: 1.9×10^8 ($1.6 - 3.7 \times 10^8$)], **B.** GLUT2 [T0: critically ill: 7.4×10^7 ($2.8 \times 10^7 - 1.2 \times 10^8$) vs. healthy: 1.5×10^8 ($3.7 \times 10^7 - 3.8 \times 10^8$)] and **C.** T1R2 (at T0: critically ill: 2.2×10^4 ($2.0 \times 10^3 - 1.9 \times 10^5$) vs. healthy: 1.5×10^5 ($4.2 \times 10^4 - 5.6 \times 10^5$)] transcript in duodenal biopsies from healthy subjects and critically ill patients.

Expression of **D.** SGLT-1 [CLP: 0.645 (0.307 – 1.237) vs. sham: 1.421 (0.683 – 2.303); $P < 0.001$], **E.** GLUT2 [CLP: 0.075 (0.032 – 0.054) vs. sham: 0.151 (0.089 - 0.237)] and **F.** T1R2 [CLP (x 10^{-4}): 0.260 (0.020 – 0.792) vs. sham: 0.831 (0.146 – 3.006)] relative to β-actin in sham and or cecal ligation and puncture (CLP) and resuscitated mice; # $P < 0.01$, § $P < 0.001$.

Figure 3. Carbohydrate malabsorption in the critically ill and CLP resuscitated mice.

A. 3-O-methylglucose (3-OMG) Area Under Curve (AUC_{30}) in humans (critically ill: 0.48 (0.14 – 2.20) vs. healthy: 1.40 (0.49 – 3.11) mmol/L.min), and **B.** 3-OMG at T30 in mice (CLP: 0.22 (0.17 – 0.73) vs. sham: 0.74 (0.46 – 1.56) mmol/L); # $P < 0.01$.

Figure 4. Cecal ligation and puncture is associated with increased morbidity and mortality in resuscitated mice. A. Survival rates in CLP and sham-operated mice **B.** Cumulative Disease Index score in CLP and sham-operated mice (CLP: 18.8 ± 2.4 vs. sham: 4.8 ± 0.5); * $P < 0.05$.

Figure 5.Sweet taste cells express GLP-1 and GIP in human duodenum. Immunolabelling for T1R2 was present in of **A.** 19 ± 11% of L-cells and **B.** 15 ± 10% of K-cells in the duodenum of healthy subjects. **C.** SGLT-1 immunolabelling was evident in the brush border membrane of duodenal enterocytes, while **D.** GLUT2 labeled within basolateral membranes in healthy subjects. Intestinal lumen is to the top of C, D. Scale Bar (A-B) = 20 µm, (C, D) = 50 µm.

TABLES

Variable	Critically Ill	Healthy Controls
Age, years	46 (33 - 76)	36 (24 - 78)
Gender, n		
Males	10	11
Hb_{A1c}, %	5.7 (5.1 - 6.4)	5.7 (4.8 - 6.4)
Body mass index, kg/m^2	27 (20 - 35)	24 (20 - 39)
Admission Diagnosis, n		N/A
Traumatic injuries	4	
Pneumonia	4	
Traumatic injury complicated by nosocomial pneumonia	2	
Meningitis	1	
Subarachnoid hemorrhage	1	
Length of ICU admission before study, days	4.5 (2 - 10)	N/A

Acute Physiology and Chronic Health Evaluation (APACHE) II score		N/A
Admission	22 (5 - 29)	
Study Day	19 (7 - 26)	
Receiving intravenous norepinephrine during study, n	2	N/A

Table 1: Characteristics of the human cohort

Data are median (range)

Variable	Critically Ill	Healthy Controls
Insulin T0 (mU/L)	7 (1 - 49)	5 (2 - 17)
Insulin T30 (mU/L)	17 (1 - 69)	39 (8 - 70)
Insulin increment (mU/L)	12 (0 - 42)	35 (3 - 68) *
Glucagon T0 (pg/mL)	97 (51 – 352)	55 (47 – 143) *
Glucagon T30 (pg/mL)	106 (55 – 260)	55 (37 – 145) *
GLP-1 T0 (pmol/L)	42 (19 – 137)	18 (11 – 42) *
GLP-1 T30 (pmol/L)	50 (22 - 171)	35 (20 - 62)
GLP-1 increment (pmol/L)	10 (-10 - 95)	19 (-10 - 41)
GIP T0 (pmol/L)	13 (6 - 36)	14 (10 - 23)
GIP T30 (pmol/L)	32 (19 - 44)	28 (15 - 46)
GIP increment (pmol/L)	19 (4 - 23)	23 (2 - 28)

Table 2 Insulin, glucagon, GLP-1 and GIP concentrations in the critically ill and in health

GLP-1 (glucagon-like peptide-1); GIP (glucose-dependent insulinotropic polypeptide); increment = T30-T0; * Critically ill patients when compared to healthy subjects $P < 0.05$; Data are median (range).

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