# ENDOPLASMIC RETICULUM STRESS INCREASES GLUCOSE PRODUCTION IN VIVO VIA EFFECTS ON LIVER GLYCOGENOLYSIS AND GLUCOSE-6-PHOSPHATASE ACTIVITY

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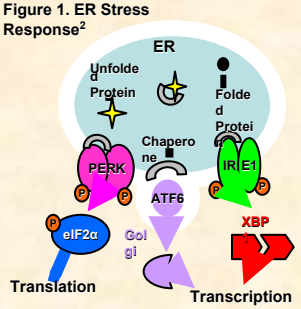
**Abstract:** Recent evidence suggests that endoplasmic reticulum (ER) stress can induce impairments in both insulin secretion and insulin action. The aim of the present study was to examine the effects of ER stress on glucose production in vivo. Fasted rats were anesthetized and catheters were placed in the carotid artery, jugular vein, and jejunal vein. A pancreatic clamp was performed in which somatostatin was infused to inhibit pancreatic insulin and glucagon secretion. These hormones were then replaced at basal levels. To examine the effects of ER stress on glucose production, 6,6-$^2H_2$ Glucose was infused in the absence (CON, n =4) or presence of jejunal vein tunicamycin delivery (TUN, n =6). TUN induces ER stress through inhibition of protein glycosylation. Arterial insulin, glucagon, corticosterone, and free fatty acid concentrations were constant throughout experiments and were not different between groups. Glucose concentration and production increased by 76.2±24.2 mg/dl and 2.6±1.2 mg/kg/min (mean±SDEV), respectively, in TUN, but did not change in CON. Liver glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase mRNA were not different between groups. Liver, but not kidney, G6Pase activity (nmoles/mg protein/30min) was increased in TUN (7.2±2.1) vs. CON (0.2±0.3). Liver glycogen concentration was reduced by 62% in TUN vs. CON. These data suggest that experimental induction of ER stress can increase the production of glucose in vivo, in part, via activation of hepatic glycogenolysis and G6Pase.

**Background:** The Endoplasmic Reticulum (ER) has an important role in balancing protein load and protein folding. Pathological stresses can disrupt homeostasis, leading to the accumulation of unfolded proteins, which are toxic to the cell. This imbalance, or "ER stress," leads to an "ER stress response" (Figure 1). This response activates three membrane bound proteins which ultimately leads to the acute inhibition of protein translation and increased transcription of chaperone proteins and proteins involved in protein degradation[1].

**Figure 1. ER Stress Response[2]**




**Aim:** Recent evidence suggests that the ER stress response can lead to impairments in insulin secretion and insulin resistance. Type 2 diabetes is characterized by impairments in insulin secretion, insulin resistance, and overproduction of glucose by the liver. The aim of this study was to examine the effects of ER stress on glucose production. We hypothesized that ER stress would increase glucose production.

**Methods:** Male rats were 4-8 hours fasted. Rats were anesthetized with 50 mg/kg of sodium pentabarbitol. Catheters were placed in the carotid artery (blood sampling), jugular vein (infusions), and jejunal vein (treatment). Experiments were 90 minutes in duration.

**Isotope Dilution:** 6,6 $^2H_2$- Glucose was infused for the duration of the experiment to estimate glucose production.

**Pancreatic Clamp Technique:** In all rats, somatostatin was infused (2 µg/kg/min) to inhibit pancreatic insulin and glucagon secretion. Insulin and glucagon were then replaced at basal levels.

**Treatment:** Six rats were infused with tunicamycin (inhibits protein glycosylation to induce ER stress) as the treatment group. Four rats were infused with saline as the control group.

**Sampling:** Blood samples were taken throughout the 90 minute experiment. A liver sample was taken prior to and following experiments. A kidney sample was taken following experiments.

**Methods Cont.**

**G6Pase:** G6Pase activity was determined on whole liver homogenates at G6P concentrations of 0, 2.5, and 10 mM as described by Nordlie and Arion[4].

**Glycogen:** Was determined on liver homogenates by alkaline hydrolysis and alcohol precipitation[5].

**Real-Time PCR:** PCR was performed on transcribed cDNA using IQ-SYBR green master mix (Bio-Rad) and primer sets designed by the Beacon designer program version 3.1.

**Analysis:** Plasma levels of glucose, free fatty acids, corticosterone, glucagon, and insulin were measured using standard techniques.

**Figure 2. Experimental Design**

**Infusions:** (*, Jugular Vein; *, Portal Vein)

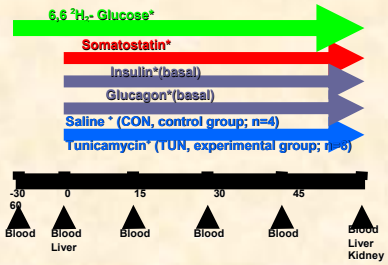
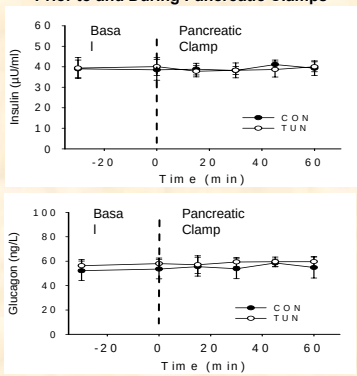
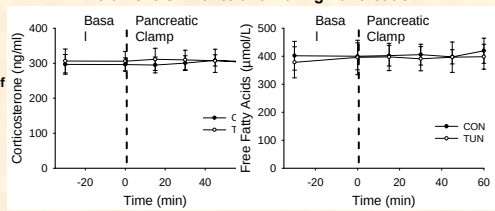



**Figure 3. Plasma Insulin and Glucagon Levels Prior to and During Pancreatic Clamps**



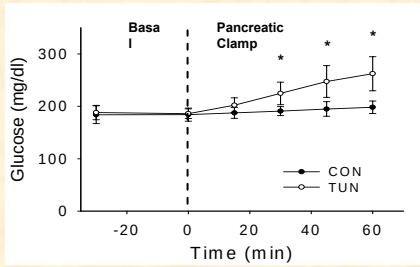

Values are mean±SDEV for both TUN and CON groups. TUN, n=6; CON n=4.

**Figure 4. Plasma Corticosterone and Free Fatty Acid Levels Prior to and During Pancreatic**




Values are mean±SDEV for both TUN and CON groups. TUN, n=6; CON, n=4.

**Figure 5. Glucose Levels Prior to and During Pancreatic Clamp**

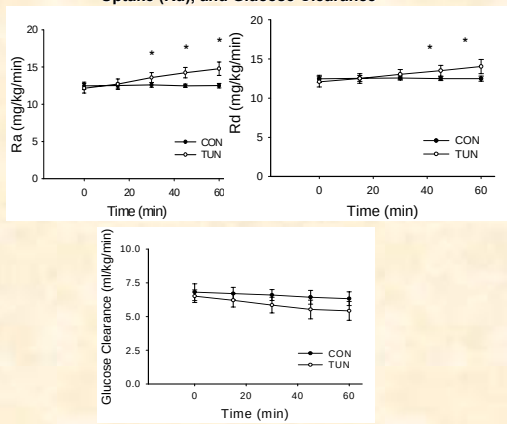



Values are mean±SDEV for both TUN and CON groups. TUN, n=6; CON, n=4. *, significantly different from CON (p<0.05)

With the increase in glucose levels, we then examined whether this was due to an increase in glucose production, decrease in glucose removal, or a combination of both.

**Glucose Production and/or Removal**

**Figure 6. Glucose Production (Ra), Glucose Uptake (Rd), and Glucose Clearance**




Values are mean±SDEV for both TUN and CON groups. TUN, n=6; CON, n=4. *, significantly different from CON (p<0.05)

Increased glucose production in response to Tunicamycin could result from:

1. Increased expression of genes/proteins involved in glycogenolysis, gluconeogenesis, and/or glucose release.

2. Acute activation of glycogenolysis, gluconeogenesis, and/or glucose release.

Real-Time PCR analysis of phosphoenol pyruvate carboxykinase (PEPCK, a rate limiting protein in gluconeogenesis) and glucose-6-phosphatase (G6Pase, responsible for glucose release from hepatocyte) demonstrated that these two genes were not increased over the time course of the experiment.

We next investigated whether TUN activated glucose-6-phophatase, an ER localized enzyme responsible for dephosphorylation of glucose-6-phosphate to glucose.

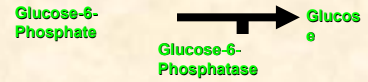
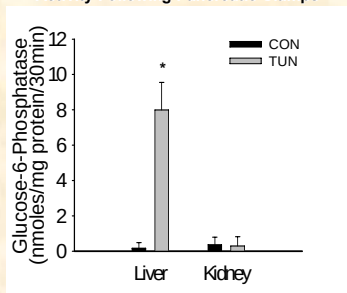


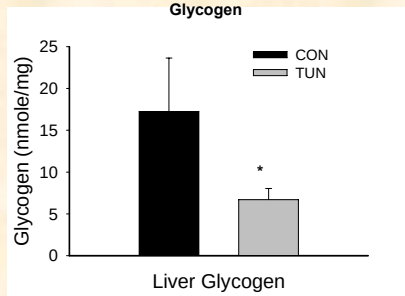

**Figure 7. Glucose-6-Phosphatase Activity Following Pancreatic Clamps**




Values are mean±SDEV for both TUN and CON groups. TUN, n=4; CON, n=4. *, significantly different from CON (p<0.05)

To determine whether the supply of glucose-6-phosphate might also be increased we measured liver glycogen concentrations in terminal liver samples.

**Figure 8. Terminal Liver Glycogen**




Values are mean±SDEV for both TUN and CON groups. TUN, n=4; CON, n=4. *, significantly different from CON (p<0.05)

**Summary:** These data suggest that experimentally induced ER stress increased glucose production in vivo. The data also suggest that the increase in glucose production was due, in part, to an increase in hepatic glucose-6-phosphatase activity, and perhaps increased hepatic glycogenolysis.

**Relevant References**

1. Ozcan, U. et al. *Science* 306: 457-461, 2004.
2. Rutkowski & Kaufman *TRENDS in Cell Biology*, 14.1:20, 2004
3. Wang, D. et al. *Endocrinology*, 147:350-358,2006
4. Nordlie RC and Arion WJ. *Methods Enzymol* 9: 619-625, 1966
5. Good, C. et al. *J. Biol. Chem.* 100:485-491, 1933