Hepatic pyruvate carboxylase expression and activity in a diabetic model rat

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Background: Hepatic gluconeogenesis is responsible for fasting hyperglycemia in type 2 diabetes mellitus (T2DM); however, the mechanism by which gluconeogenesis is increased is unclear. Currently, this is largely attributed to increased expression of PEPCK and glucose-6-phosphatase. This hypothesis was supported by rodent models that had increased glucocorticoid (e.g. ZDF rat), which is not a feature of human T2DM. Recent studies demonstrated that fasting hyperglycemia can manifest without increased hepatic expression of PEPCK or G6Pase both in rat models of T2DM (without increased glucocorticoids) as well and in humans with T2DM. Thus, other mechanisms must account for hyperglycemia.

Hypothesis: Fasting hyperglycemia and increased gluconeogenesis in T2DM is attributable to increased activity of pyruvate carboxylase, which catalyzes the first step in gluconeogenesis.

Specific Aims: 1. To determine whether PC expression and PC activity are increased in diabetic rats. 2. To determine whether PC expression can be regulated by islet hormones (insulin and glucagon).

Methods: 1) We examined hepatic PC mRNA and protein expression from nicotinamide/streptozocin treated, high-fat fed rats (STZ/HFF), a rat model of T2DM where fasting hyperglycemia develops with inappropriately normal insulin and glucagon levels as in patients with T2DM. Fresh frozen rat livers were homogenized, and mitochondria isolated by centrifugation in sucrose. Concentrations of mitochondria were normalized by dilution in mitochondrial isolation buffer after measurement of total protein by the Bio-Rad/Bradford assay. Mitochondria disrupted by brief sonication. PC activity determined by a coupled assay, PC activity coupled to malate dehydrogenase (MDH), with pyruvate, MDH, and NADH in excess. Decay in NADH concentration was followed at 340 nm, multiple simultaneous assays performed in a multiwell spectrophotometer mRNA expression was determined by real time PCR. Relative PC protein levels determined by Western blot. 2) To assess the roles of insulin and glucagon in regulating PC expression, primary hepatocytes were isolated from SD rats fasted overnight. Following recovery, hepatocytes were then exposed for four hours to insulin 0, 1 and 100 nM, glucagon 0, 0.1 and 10 nM. After 4 hours, cells were isolated into Rneasy RLT buffer for cDNA isolation.

Results: As the STZ/HFF treatment produces variable degrees of fasting hyperglycemia, the rats used in this study were stratified by degree of hyperglycemia (control: 119±4 mg/dl; moderately hyperglycemic STZ/HFF: 151±6mg/dl; very hyperglycemic STZ/HFF: 443±14mg/dl). As compared with control rats, PC protein was increased by 76±10% (P<0.001) in the very hyperglycemic group of STZ/HFF rats. We adopted a spectrophotometric assay for PC activity from the literature, and optimized it for use with our rat liver mitochondria preparation. PC activity was increased by 77±16% (P<0.005) in the very hyperglycemic STZ/HFF rat livers vs. control rat livers. In contrast with the protein and activity data, PC mRNA was not significantly different between the control and STZ/HFF rat livers. The effect of insulin and glucagon on PC expression was examined in primary culture. PC expression in rat hepatocytes was suppressed by insulin and increased by glucagon, both in a dose-dependent manner.

Conclusions: While in tissue culture we saw that insulin and glucagon could regulate PC gene expression, in contrast, in the STZ/HFF rat, PC protein and activity were increased without an increase in mRNA transcripts. Future studies will focus on post-translational modifications of the PC protein, looking for modifications that affect the half-life of the protein (eg ubiquitination), and particularly searching for modifications downstream of insulin and glucagon signaling pathways. Additionally, we will attempt to identify conditions in tissue culture that demonstrate divergence between protein and mRNA levels, similar to what we see in the STZ/HFF model.