ISIS PHARMACEUTICALS AND ITS COLLABORATORS AT THE AMERICAN DIABETES ASSOCIATION'S 71ST SCIENTIFIC SESSIONS JUNE 24-28, 2011 SAN DIEGO, CA

Session Type	Poster Presentation
Session Title	General Poster Session II – Obesity-Animal
Poster #	1822-P
Location	Hall B
Date	**Sunday, June 26, 2011**
Time	**12:00 p.m. – 2:00 p.m. P.T.**
Presentation	**ANTISENSE REDUCTION OF FGFR4 EXPRESSION: A NOVEL THERAPEUTIC APPROACH FOR OBESITY**

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We recently reported that antisense reduction of FGFR4 expression lowered adiposity and improved insulin sensitivity and that it also augmented appetite suppressant-induced anti-obesity effect in rodents (Yu et al, Diabetes, Suppl 1, A83, 2009 & A474, 2010). To expand these findings, we evaluated the anti-obesity effect of FGFR4 inhibition in DIO mice with caloric restriction (CR). Specifically, DIO C57BL/J mice fed a 58% fat diet *ad lib* were treated with saline, a control (CTL) ASO or FGFR4 ASO (R4 ASO) at 25 mg/kg twice weekly. After 2 wk of treatment (Rx), they were switched to CR by providing 95% of the amount of food consumed daily by R4 ASO group during the first 2 wk of Rx. The Rxs were continued for another 4 or 6 wk. In both studies, R4 ASO reduced liver FGFR4 mRNA levels by 75-80% whereas CTL ASO had no effect. CR significantly decreased both BW and body fat content (BF) of saline-treated group with CR (saline-CR) as compared to saline-treated group fed *ad lib*. CTL ASO did not change BW or BF versus saline-CR. However, R4 ASO further lowered BW by 7-11% and BF by 23-25%. In addition, R4 ASO reduced epididymal fat pad wt by 16-22% and peri-renal fat pad wt by 29-33% without effect on lean mass. As expected, CR significantly reduced whole body VO_2. In contrast, R4 ASO prevented the decline in VO_2. In separate studies, R4 ASO Rx of lean C57BL/J and CD-1 mice for 3 mo resulted in > 75% reduction in liver FGFR4 mRNA and was well tolerated without any overt side effects, indicating lack of mechanism based toxicity with chronic reduction of FGFR4. To further explore mechanism of action, the effect of R4 ASO Rx on FGF15 levels was determined. Four-wk Rx of DIO mice reduced liver FGFR4 mRNA by > 75%, increased ileum FGF15 mRNA by > 5-fold and plasma FGF15 protein levels by > 2-fold. These rodent findings were confirmed in Cynomolgus monkeys, where Rx with R4 ASO for 3 mo reduced liver FGFR4 mRNA by 70% and caused > 5-fold increase of ileum FGF19 mRNA and 2-fold increase of plasma FGF19 protein levels without any overt toxicities. These data provide further support that peripheral inhibition of FGFR4 with antisense drugs is an attractive therapeutic approach for obesity.

Session Type	Poster Presentation
Session Title	General Poster Session II – Integrated Physiology-Adipocyte Biology
Poster #	1592-P
Location	Hall B
Date	**Sunday, June 26, 2011**
Time	**12:00 p.m. – 2:00 p.m. P.T.**
Presentation	**ANTISENSE OLIGONUCLEOTIDE KNOCKDOWN OF PEPCK PROTECTS AGAINST FAT INDUCED OBESITY BUT RESULTS IN HEPATIC STEATOSIS AND HEPATIC INSULIN RESISTANCE**

Sara A. Beddow, Sachin Majumdar, Katarina Topchyan, Gary W. Cline, Prasad Manchem, Brett P. Monia, Sanjay Bhanot, Gerald I. Shulman, Varman T. Samuel

Phosphoenolypyruvate carboxykinase (cytosolic form, PCK1), a key gluconeogenic enzyme, also supports glyceroneogenesis for adipocyte fat storage. To determine if PCK1 is a viable therapeutic target for T2DM, we used antisense oligonucleotides (ASO's) to decrease PCK1 expression in liver and white adipose tissue (WAT) in hyperglycemic, streptozotocin-treated/high-fat fed rats (STZ/HFF). Compared to a Control ASO, PCK1 ASO decreased PCK1 expression by ~80%. While this failed to correct the hyperglycemia, there was a trend towards decreased adiposity. To better assess this effect, normal rats were fed either a control (C), high-fructose (HFr) or HF diets. In the C and the HFr groups, PCK1 ASO had no significant effects (though PCK1 ASO reduced plasma TG by 33% in HFr group). In contrast, in HFF rats PCK1 ASO decreased body weight ~30%, with a ~50% decrease in epididymal WAT mass. Whole body calorimetery in ASO treated, HFF C57BL/6 mice demonstrated that the decrease in adiposity develops without a decrease in caloric intake or increase in energy expenditure. Insulin sensitivity was assessed with euglycemic-hyperinsulinemic [4 mU/(kg-min)] clamps in HFF rats. Fasting plasma glucose and insulin concentrations and basal rates of endogenous glucose production (EGP) were unaltered. However, PCK1 ASO treated rats were insulin resistant, with a 32% reduction in the glucose infusion rate required for euglycemia (CONT: 22.9±1.9 vs. PCK1: 15.6±1.4, P=0.01). This is attributed to both a 22% decrease in whole-body insulin-stimulated glucose disposal (P=0.04) and a failure to suppress EGP (60±6% vs. 35±6%, P=0.02). The development of hepatic insulin resistance was associated with a 75% increase in liver TG content (P=0.02). **CONCLUSIONS**: Decreasing adipose PCK1 expression prevented fat-induced adiposity but promoted ectopic lipid deposition and hepatic and peripheral insulin resistance in fat-fed rats. Despite knockdown of hepatic PCK1, fasting plasma glucose concentrations were unaffected under multiple conditions. These data demonstrate a key role for PCK1 dependent glyceroneogenesis for the storage of dietary fat in adipocytes.

Session Type	Poster Presentation
Session Title	General Poster Session III – Integrated Physiology-Liver
Poster #	1693-P
Location	Hall B
Date	**Monday, June 27, 2011**
Time	**12:00 p.m. – 2:00 p.m. P.T.**
Presentation	**PYRUVATE CARBOXYLASE, A NOVEL THERAPEUTIC TARGET FOR TYPE 2 DIABETES**

Naoki Kumashiro, Sara A. Beddow, Ioana Fat, Sachin K. Majumdar, Fitsum Guebre-Egziabher, Jennifer L. Christianson, Prasad Manchem, Brett P. Monia, Sanjay Bhanot, Gerald I. Shulman, Varman T. Samuel

Fasting hyperglycemia in T2D is due to increased gluconeogenesis, a process for which the enzymatic regulation is poorly understood. We recently observed an association between fasting hyperglycemia and expression of pyruvate carboxylase (PC) but not PEPCK and G6Pase in several rodent models and thus, hypothesized that PC is an excellent therapeutic target for T2D. To test this, we used antisense oligonucleotides (ASO's) to decrease PC expression in normal SD rats fed a regular chow, high-fat fed (HFF) SD rats and Zucker Diabetic Fatty (ZDF) rats. ASO's specifically decrease target expression in liver and adipose but not other tissues (e.g. pancreas). PC ASO treatment decreased PC expression by ~90%. In regular chow fed rats, this reduced plasma glucose concentrations in both the fasted [Cont: 109±1 vs. PC: 103±1, (P<0.01)] and *ad lib* states [146±2 vs. 133±3 mg/dl (P<0.01)], without ketosis or lactic acidosis**.** Plasma insulin concentration was unchanged when fasting, but surprisingly, increased in the *ad lib* fed state [27±2 vs. 47±7 µU/mL (P<0.01)]. In HFF rats, PC ASO reduced fasting plasma glucose [125 vs. 108 mg/dl (P<0.01)], fasting insulin [12.9±1.5 vs. 8.5±1.2 µU/mL (P<0.05)] and HOMA-IR (4.0±0.5 vs. 2.3±0.4 mg/dl*µU/mL (P<0.01)]. The changes in insulin sensitivity were assessed with a euglycemic-hyperinsulinemic (4 mU/kg-min) clamp. Both basal endogenous glucose production (EGP) and clamped EGP were reduced 18% (P<0.01) and 33%, respectively. Interestingly, PC ASO reduced epididymal fat mass 25% and hepatic triglyceride content 33% (both P<0.05). In ZDF rats, while PC ASO had no effect on body weight or adiposity, the effects on glucose persisted, with reductions in fasting glucose [168±6.7 vs. 149±5.3 mg/dl (P<0.05)], basal EGP [9.3±0.9 vs. 6.9±0.4 mg/kg-min (P<0.05)] and clamped EGP [4.0±0.5 vs. 2.7±0.1 mg/kg-min (P<0.05)]. In contrast, insulin–stimulated peripheral glucose disposal was unchanged in both models. **CONCLUSION:** ASO mediated inhibition of PC *in vivo* is a novel therapeutic approach that safely and effectively lowers plasma glucose in a variety of rodent models by reducing hepatic glucose production and surprisingly augmenting postprandial insulin concentrations.