Development of an In-vitro Model of Hepatic Steatosis Using Rat Hepatocytes Under Controlled Hemodynamics in a Diabetic Milieu.

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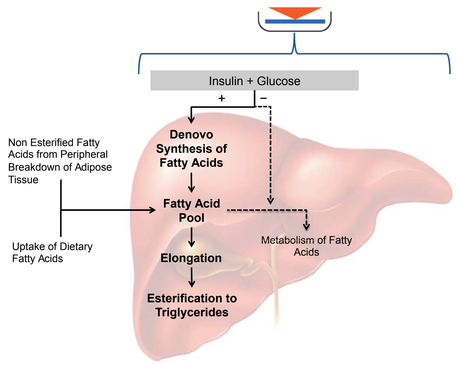
ABSTRACT

Hepatic steatosis (excessive lipid accumulation in the liver), is correlated with obesity and type II diabetes, leading to steatohepatitis and cirrhosis, if untreated. Though animal steatosis models use a low fat/high carbohydrate diet to stimulate lipogenesis, corresponding in vitro hepatocyte models lack an adequate insulin-glucose response to induce the same, probably on account of the superphysiological levels of insulin/glucose required to maintain hepatocytes in culture. We recently showed that hepatocytes cultured in the presence of liver-derived hemodynamics, retained differentiated function and response at physiological glucose and insulin levels. Using this system, we tested the hypothesis that introducing a high insulin/glucose 'disease' milieu would induce fatty changes. Primary rat hepatocytes were cultured in the system for 7 days using medium containing elevated levels of glucose and insulin representing a 'disease milieu' or as 'healthy' controls with near physiological levels of these factors, before harvesting for endpoint assays. Pioglitazone, a drug used to treat steatosis, was tested in the system. Oil red O quantification indicated total lipid was raised in disease milieu cultures under flow (~3 fold higher than healthy) and confirmed by Nile Red staining. Total triglycerides were also increased under the disease conditions. RT-PCR demonstrated glycerol-3-phosphate acyltransferase (GPAT) and sterol regulatory element-binding protein (SREBP), key genes responsible for lipogenesis, were induced under the diabetic milieu relative to the healthy condition. The steatotic changes were accompanied by concomitant metabolic changes. Under hemodynamic flow, hepatocytes under healthy media conditions maintained high levels of mRNA expression of Cyp1a1, Cyp 2b1, 2b2, Cyp3a2,and (20, 90, 30 and 40-fold higher than traditional static cultures respectively), whereas Cyp 2b2 and Cyp 3a2 levels in hepatocytes cultured in the fatty liver media were decreased by 9 and 12 fold compared to healthy. Activity of CYP3A2 and CYP1A1 was also reduced 3- 6 fold in diabetic conditions compared to 'healthy', as measured by p45glo assay. Pioglitazone was effective in reducing the lipid and triglyceride content while restoring metabolic gene expression under the disease conditions. In summary, we adapted a system that preserves in vivo-like hepatocyte phenotype and response, to create a model of hepatic steatosis by inducing pathological steatotic changes in the presence of a high glucose/insulin milieu. The fatty changes appear to be mediated by an upregulation of de novo lipogenesis and are associated with a concomitant loss of metabolic function. The system successfully demonstrates the effect of pioglitazone in preventing the steatotic changes.

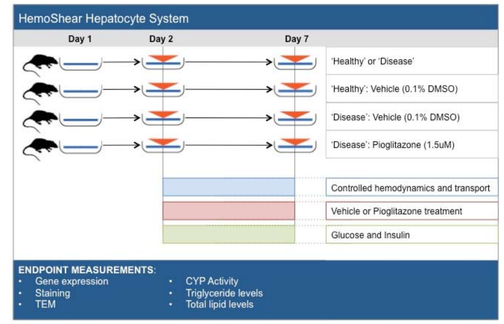
BACKGROUND

- Non Alcoholic Fatty Liver Disease (NAFLD) is correlated with obesity, type II diabetes and metabolic syndrome in the presence of insulin resistance and is characterized by hepatic steatosis that if untreated progresses to inflammatory changes (steatohepatitis) and ultimately cirrhosis[1].
- Animal models induce steatosis through a hyperglycemic-hyperinsulinemic environment but current in vitro models fail to induce fatty changes in hepatocytes through insulin and glucose, perhaps due to impaired insulin responsiveness of hepatocytes under static culture conditions and rapid dedifferentiation hepatocytes are known to undergo in vitro.
- We have previously described a system that uses controlled hemodynamics and transport to retain and restore primary hepatocyte phenotype, morphology and function over time[2].
- Hepatocytes in this system display induction and toxicity responses to various drugs tested in this system at concentrations much closer to in vivo and clinical C_{max} levels than current static culture systems[3].
- We hypothesized that controlled hemodynamics and transport could similarly reproduce a more physiological response to insulin and glucose in hepatocytes maintained under controlled hemodynamics, thereby inducing the fatty changes associated with steatosis in a hyerinsulemic, hyperglycemic environment as typically seen initially under insulin resistant conditions of diabetes

LIPID REGULATION IN LIVER IS A COMPLEX AND DYNAMIC PROCESS



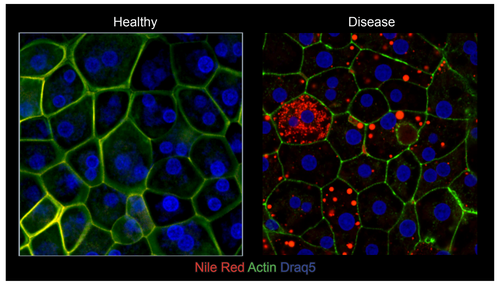
EXPERIMENTAL OUTLINE



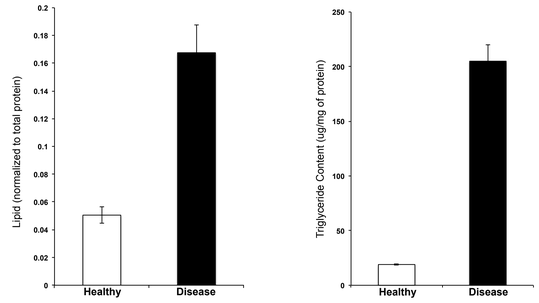
HemoShear Hepatocyte System Media Components

Component	'Healthy'	'Disease'
Glucose	5.5 mM 100 mg/dL	17.5 mM
Insulin	2 nM 2000 pmol/L	2 uM 2 x 10^6 pmol/L
Dexamethasone	100nM	100nM

INCREASED GLUCOSE AND INSULIN INDUCE LIPID ACCUMULATION IN HEPATOCYTES UNDER HEMODYNAMIC CONDITIONS

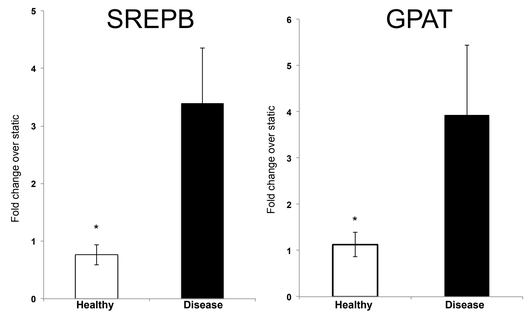


Rat hepatocytes cultured under high glucose/insulin 'disease' conditions develop lipid droplets as measured by Nile Red staining.



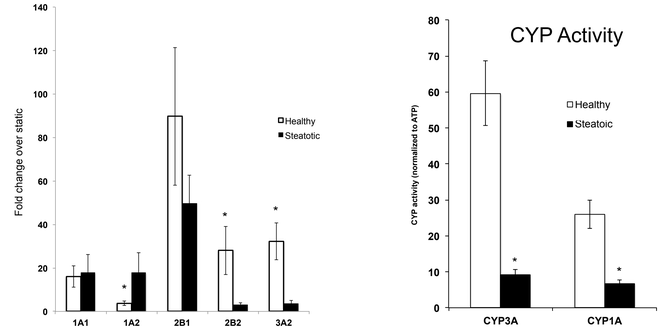
Total lipid and triglycerides are increased in hepatocytes cultured under high glucose and insulin. (n=3)

KEY LIPOGENESIS GENES ARE REGULATED BY INSULIN AND GLUCOSE



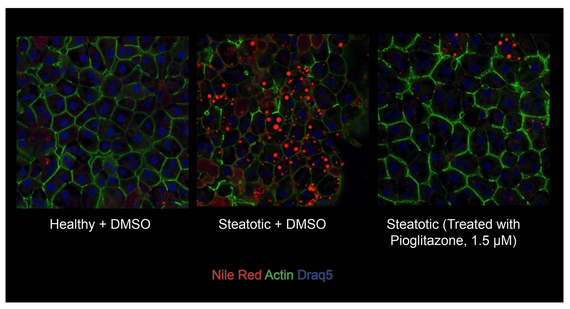
High glucose/insulin treated rat hepatocytes have increased mRNA levels of the transcription factor sterol regulatory element-binding protein (SREBP) and glycerol-3-phosphate acyltransferase (GPAT) gene which regulates genes involved in lipid synthesis. (* $p<0.05$, $n>5$)

GLUCOSE AND INSULIN ALTER CYP GENE EXPRESSION AS WELL AS ACTIVITY UNDER HEMODYNAMIC FLOW

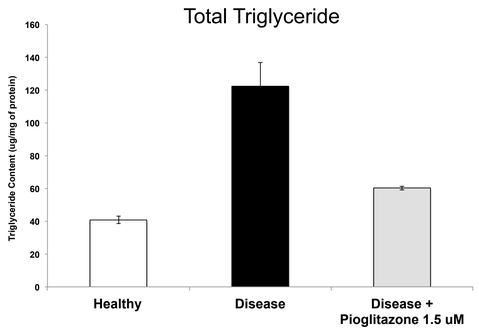


mRNAs for phase I enzymes involved in drug metabolism are differentially regulated under low and high glucose/insulin conditions. Activity of Cyp3A and 1A were also decreased under steatoic conditions.(* $p<0.05$, $n>5$)

PPARγ AGONIST DECREASES LIPID ACCUMULATION IN HEMODYNAMIC STEATOTIC MODEL

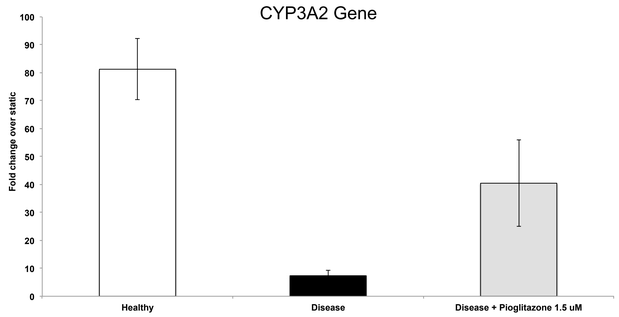


Treatment with Pioglitazone at in vivo therapeutic concentrations decreases lipid droplet formation under steatotic conditions.



Pioglitazone decreases total triglyceride content of hepatocytes under steatoic conditions to levels similar to those seen in healthy conditions. (n=3)

PIOGLITAZONE RESTORES CYP EXPRESSION AND ACTIVITY IN STEATOTIC HEPATOCYTES



Pioglitazone is able to restore the expression of metabolic genes depressed by high glucose/insulin 'disease' conditions. (n=3)

CONCLUSIONS

- Rat hepatocytes under controlled hemodynamics retain their response to insulin and glucose.
- Hepatocytes cultured under hemodynamic flow develop steatotic changes when cultured in high glucose and insulin ('disease') conditions.
- The steatosis is mediated via de novo lipogenesis with upregulation of two key genes (SREBP and GPAT).
- The increase in lipid accumulation and triglyceride content is accompanied by a concomitant decrease in metabolic gene expression and activity.
- Treatment with PPAR-γ agonist pioglitazone helps prevent the buildup of lipid and loss of metabolic activity under the high glucose and insulin conditions.
- These data demonstrate a novel and important new in vitro model of diet induced non-alcoholic fatty liver disease (NAFLD) for which none currently exist.

ACKNOWLEDGEMENTS

- Authors acknowledge Caroline Languasco, Nathan Day, and Svetlana Markurian for their contributions to the experimental work.
- This work was supported in part by NIH SBIR (R44DK_091104_R43)