Enteral insulin attenuates fatty liver signs and symptoms in mice on a high-fat diet

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BACKGROUND

Nonalcoholic fatty liver disease (NAFLD) is now the most common liver disorder in the USA, affecting 90% of morbidly obese individuals and often clustering with prediabetes or overt type 2 diabetes mellitus. Approximately 40% of NAFLD patients progress to nonalcoholic steatohepatitis (NASH), the most common cause of cryptogenic cirrhosis, which subsequently can to exacerbate to advanced fibrosis. These patients show increased risk of hepatocellular carcinoma and cardiovascular and liver-associated mortality. While its exact pathogenesis remains unclear, insulin resistance and obesity are considered contributing and risk factors, suggesting effective blood glucose control as a means of preventing and managing NAFLD.

OBJECTIVE

- To establish a proof-of-concept for the therapeutic value of oral insulin in counteracting inflammatory processes associated with chronic liver disease

METHODS

C57b1/6J mice (n=33) were maintained on a high-fat diet (HFD) for 16 weeks, and were administered daily insulin (INS; 3.6 mg/kg), delivered via a duodenal feeding tube (n=18), or sham-saline treatment (n=15) during the last three weeks. At the end of the treatment period, peripheral blood and liver biopsy samples were collected to analyze inflammatory and fibrosis markers and to histologically assess NAFLD activity score (NAS). FACS analysis was performed on harvested mouse liver lymphocytes ($1x10^6$/ml) incubated with antibodies specific to Pan leukocyte (CD45-Pe Cy7), Pan T cell (CD3-APC), natural killer (NK) cell (NK1.1-Percp-Cy5), NK cytotoxicity (LAMP-1) or NK cell activity (NKp46) markers. Real time PCR was performed to quantify α-smooth muscle actin (α-SMA) expression.

RESULTS

Mice on the HFD diet became exceedingly obese over the study period, but the feeding tube seemingly disrupted eating patterns, and animals subsequently lost weight in the last two weeks of treatment. Yet, a difference between the two groups was still noted, with insulin-treated animals demonstrating a slightly larger reduction from baseline body weight as compared to the sham animals (Figure 1; 8.6±1.8g and 7.5±4.1 g, respectively, p=0.16). In addition, mean NAS scores were lower among INS-treated (0.11) as compared to PBS-treated animals (0.2).

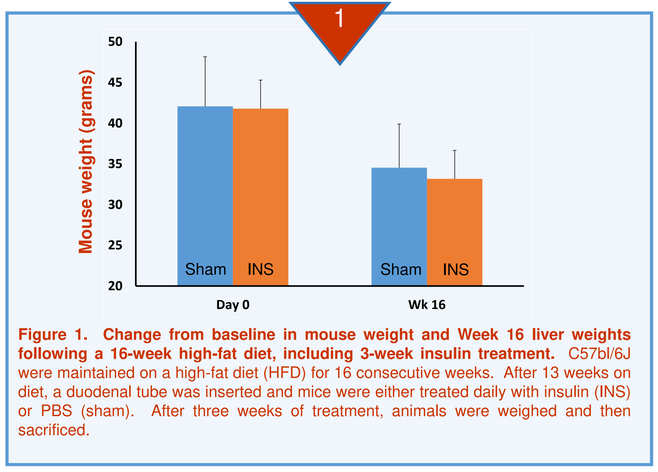

Figure 1. Change from baseline in mouse weight and Week 16 liver weights following a 16-week high-fat diet, including 3-week insulin treatment. C57bl/6J were maintained on a high-fat diet (HFD) for 16 consecutive weeks. After 13 weeks on diet, a duodenal tube was inserted and mice were either treated daily with insulin (INS) or PBS (sham). After three weeks of treatment, animals were weighed and then sacrificed.

By the completion of treatment, both sham and insulin-treated animals showed significantly lower serum ALT levels (Figure 2A; 38.3±34.5 U/L and 88.4±85.3 U/L, respectively; p=0.02). In contrast, post-treatment AST levels were 2-fold lower in the insulin treatment versus sham group (Figure 2B; 123.2±80.4 U/L and 202.8±83.7 U/L, respectively; p=0.03). The mean AST/ALT ratio, a reliable indicator of liver disease, was 4.7±2.9 in the PBS group (p=0.002) and 2.6±1.3 (p=0.009) in the INS group (Figure 2C).

Sham animals showed higher change from baseline α-SMA RNA levels (0.58±0.14-fold) as compared to those treated with insulin treatment (0.31±0.23-fold) (Figure 3A). In parallel, a consistently higher percentage of active ($NKP46^+$) $CD3^+$ natural killer (NK) cells was detected in insulin-treated versus sham-treated mouse livers (Figure 3B).

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Figure 3. α-SMA transcript levels and inflammatory cell activity following an 16-week high-fat diet + insulin regimen. C57bl/6J were maintained on a high-fat diet (HFD) for 16 consecutive weeks. After 13 weeks on diet, a duodenal tube was inserted and mice were either treated daily with insulin (INS) or PBS (Sham). (A) RT-PCR analysis of α–SMA levels. (B) Active natural killer profiles were assessed by FACS analysis.

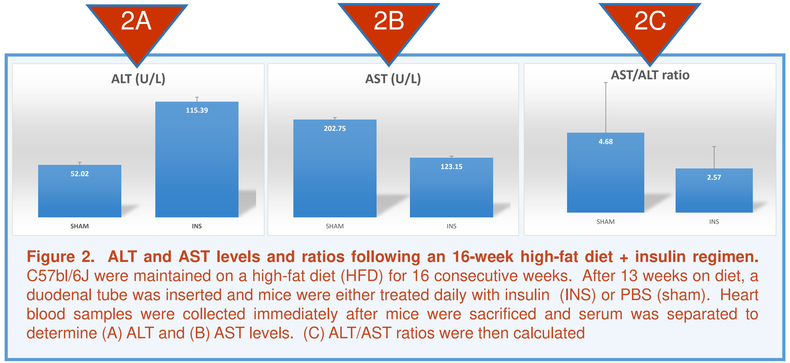

Figure 2. ALT and AST levels and ratios following an 16-week high-fat diet + insulin regimen. C57bl/6J were maintained on a high-fat diet (HFD) for 16 consecutive weeks. After 13 weeks on diet, a duodenal tube was inserted and mice were either treated daily with insulin (INS) or PBS (sham). Heart blood samples were collected immediately after mice were sacrificed and serum was separated to determine (A) ALT and (B) AST levels. (C) ALT/AST ratios were then calculated

CONCLUSIONS

While the surgical intervention impacted animal eating patterns, with consequential weight loss in both animal groups, a number of small differences were still noted following insulin treatment. INS mice lost an average of 1.1 gram more and presented lower NAS scores than sham animals. Moreover, a 2-fold decrease in AST levels was measured among INS mice only, and although ALT levels, often a hallmark of NAFLD were similarly affected in the INS and sham groups, mean AST/ALT ratios, considered a reliable indicator of liver diseases, were significantly lower among insulin-treated mice as compared to sham. In parallel, INS mice showed reduced signs of fibrosis and increased anti-fibrotic NK cell activity. In particular consistently higher levels of $NKp46^+$ cells, cells which have been associated with attenuation of metabolism-induced hepatic fibrosis, were detected in the INS versus PBS group. Taken together, these preliminary results provide a proof of concept of a potentially therapeutic effect of enteral insulin administration on fibrotic and inflammatory processes

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