LIVER AND ADIPOSE TISSUE MACROPHAGE DEPLETION IMPROVES HEPATIC INSULIN SENSITIVITY

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Background and aims: Recent attention has been brought to recruited adipose tissue macrophages (ATM) as a source for inflammatory factors causing insulin resistance in obesity. Here, we hypothesized that Kupffer cells (KC), the hepatic resident macrophages, play a role in the pathogenesis of high fat diet (HFD) induced hepatic insulin resistance.

Methods: Clodronate liposomes were injected intraperitoneally (i.p.) or intravenously (i.v.) to deplete macrophages prior to exposure to a short term HFD. ATM and KC activation was evaluated by immunohistochemistry and RT-qPCR. Insulin-activated signalling was investigated by western blot and insulin sensitivity assessed *in vivo* by hyperinsulinemic (2.5 $mU.kg^{-1}.min^{-1}$) - euglycemic clamp.

Results: HFD induced a 5-fold increase in hepatic triglycerides. Liver sections revealed enlarged $F4/80^+$ KC in close proximity to fat-laden hepatocytes with an increased proportion of $F4/80^+/CD68^+$ activated cells. Insulin resistance suggested by elevated blood glucose was confirmed by clamp study: glucose infusion rates to maintain euglycemia were strongly reduced under HFD (52 ± 4 mg $glucose.kg^{-1}.min^{-1}$) compared to chow fed controls (82 ± 5 $mg.kg^{-1}.min^{-1}$, P=0.0002). This was mainly attributable to the lack of inhibition of hepatic glucose production by insulin and related to decreased insulin-dependent phosphorylation of the insulin receptor and downstream Akt in the liver but not in skeletal muscles.

Clodronate liposomes injected i.p. depleted KC and ATM. This had no effect on steatosis but restored both insulin-stimulated phosphorylation of insulin receptor and Akt and insulin-dependent inhibition of hepatic glucose production in HFD animals (12 ± 5 vs 34 ± 1 $mg.kg^{-1}.min^{-1}$ in HFD-clodronate vs HFD, P=0.0002) close to control levels (standard chow, 0 ± 4 $mg.kg^{-1}.min^{-1}$). A selective depletion of KC, without change in ATM, was obtained by i.v. injection of clodronate liposomes. This improved by 50% insulin-dependent inhibition of hepatic glucose production (12 ± 2 $mg.kg^{-1}.min^{-1}$versus 25 ± 4 $mg.kg^{-1}.min^{-1}$ in untreated HFD, P=0.009), and improved Akt but not insulin receptor phosphorylation in response to insulin.

In conclusion, we demonstrated an early KC activation under HFD associated with steatosis and hepatic insulin resistance. Our data support that both hepatic and adipose tissue macrophage responses participate to hepatic resistance to insulin.