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The importance of leptin in mice and man

Leptin-specific mechanisms for impaired liver regeneration in *ob/ob* mice after toxic injury. Leclercq IA, Field J, Farrell GC.

Background/Aims: Profound impairment of liver regeneration in rodents with dysfunctional leptin signaling has been attributed to non-alcohol-induced fatty liver disorders (NAFLD). Our aim was to establish whether defective liver regeneration in *ob/ob* mice is a direct consequence of leptin-dependent, intracellular signaling mechanisms controlling cell-cycle regulation in hepatocytes. Methods: After exposure to a single hepatotoxic dose of (CCl(4)), the regenerative response to hepatic injury was studied in leptin-deficient *ob/ob* and control mice. The effects of leptin supplementation (100 μg kg^{-1} per day) were examined. We assessed entry into and progression through the cell cycle and activation of key signaling intermediates and transcriptional regulators. Results: CCl(4)-induced liver injury was equally severe in *ob/ob* and control mice. In leptin-deficient mice, it was associated with exaggerated activation of NF-κ B and STAT3 during the priming phase, abrogation of tumor necrosis factor (TNF) and interleukin (IL)-6 release at the time of G1/S transition, and failure of hepatocyte induction of cyclin D1 and cell-cycle entry. Leptin replacement corrected these defects in *ob/ob* mice by restoring TNF and IL-6 release and inducing cyclin D1. Hepatocytes entered S phase and progressed, as in wild-type mice, to vigorous mitosis and normal hepatic regenerative response. In *ob/ob* mice, low doses of TNF before CCl(4) also were associated with restitution of TNF release and proliferative capabilities. Conclusions: Impaired liver regeneration in *ob/ob* mice is caused by leptin deficiency. We propose that altered cytokine production in *ob/ob* mice is part of the mechanisms responsible for impaired proliferation in response to hepatic injury.

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Non-alcoholic fatty liver disease (NAFLD) is a condition affecting 14–21% of adults in Europe [1] and in Japan [2] with similar numbers estimated for the US population [3]. NAFLD is strongly associated with obesity, dyslipidemia, insulin resistance and type 2 diabetes mellitus [3]. Although the early stage of NAFLD, i.e. steatosis, is considered benign by itself, patients are at risk to progress to steatohepatitis and later to cirrhosis [3]. The reduced tolerance of fatty livers for ischemia is the reason for more severe ischemia-reperfusion injury and higher incidence of liver failure after liver resections and transplantation [4].

In recent years, the capacity of liver cells to regenerate was shown to be a critical factor in avoiding liver failure and improve survival not only after partial hepatectomy [5] but also in various experimental models of ischemia-reperfusion injury [6,7] and toxic liver injury [8,9]. Based on studies in Zucker rats and *ob/ob* mice, steatosis is considered the main reason for the impaired regeneration [10,11]. However, this conclusion is controversial because experiments with different models of steatohepatitis suggested that steatosis might not be sufficient to prevent regeneration [12,13]. Since Zucker rats and *ob/ob* mice have a defect in leptin receptor signaling and leptin synthesis, respectively, the recent paper by LeClercq et al. [14] addressed the important question whether leptin-deficiency rather than steatosis is the cause of the impaired regenerative response in *ob/ob* mice.

LeClercq et al. investigated the regenerative response after a toxic dose of CCl_4, a well-established model of toxic liver injury, in obese *ob/ob* mice [14]. The authors found that hepatocyte regeneration in *ob/ob* mice was not only delayed but was also substantially attenuated

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compared to the response in lean littermates. Interestingly, injection of mouse recombinant leptin completely restored the regenerative response in *ob/ob* mice [14]. These data strongly suggest that regeneration after toxic liver injury is dependent on leptin and was not affected by steatosis. The authors then proceeded to identify the critical event, which was affected by leptin-deficiency. Since the expression of cyclin D_1 was substantially delayed, and DNA synthesis and cell division were drastically reduced in *ob/ob* mice, the authors hypothesized that leptin may be involved in the priming phase of the response to toxic liver injury. During this transition period (G_1), differentiated hepatocytes in the G_0 state acquire the competence to respond to growth factors and prepare for DNA synthesis [5]. Cytokines such as TNF-α and interleukin-6 (IL-6), the protooncogene c-myc and the transcription factors NF-κB and STAT-3 are involved in this process [5]. There was no difference in c-myc mRNA expression between *ob/ob* and lean mice [14]. In addition, the higher NF-κB activation in *ob/ob* mice was not attenuated by leptin injection. However, nuclear translocation of STAT3 was enhanced in *ob/ob* mice after CCl_4 administration and could be attenuated to the levels of lean mice with injection of leptin [14]. Furthermore, TNF-α and to some degree IL-6 formation in the liver were suppressed in *ob/ob* mice. Again, leptin injection restored this deficiency [14]. Consequently, pretreatment with murine recombinant TNF-α corrected the reduced regeneration in *ob/ob* mice. Together, these results suggest that leptin is directly involved in promoting hepatocellular regeneration after CCl_4-induced liver injury mainly during the priming phase of regeneration. Although the critical molecular target of leptin could not be identified, the results suggest that leptin administration restored the physiological cytokine response necessary to promote cell-cycle entry. Overall, the important message of this manuscript is that leptin deficiency and not steatosis or energy deficiency, is responsible for the impaired regenerative response in *ob/ob* mice.

How this relates to regeneration in the human liver remains to be determined especially since the involvement of leptin in human hepatic steatosis and steatohepatitis is unclear. Elevated levels of leptin rather than depressed levels have been reported in obese and non-obese patients having steatosis or steatohepatitis [15–17]. In addition, patients with congenital leptin deficiencies are not reported to have enlarged livers nor NAFLD [18–21]. The reported relationship of leptin to the development of hepatic fibrosis in rodents [22] also has not been substantiated in humans where no correlation was found between leptin levels, inflammation, and fibrosis [17]. Thus, very exciting data derived from animal models where responses may not mimic the human condition must be interpreted with caution until similar findings can be documented in people.

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