A High-Fat Diet Impairs Liver Regeneration in C57BL/6 Mice Through Overexpression of the NF-κB Inhibitor, IκBα

Robert A. DeAngelis,1,2 Maciej M. Markiewski,1 Rebecca Taub, 2,3 and John D. Lambris1

Despite the growing incidence of obesity, knowledge of how this condition, as well as associated steatosis, affects liver regeneration remains scarce. Many previous studies have used models of steatohepatitis or obesity induced by genetic alterations. In contrast, our studies on liver regeneration have focused on the effects of obesity resulting solely from high amounts of fat in the diet. This model more closely reflects the detrimental effects of dietary habits responsible for increased morbidity due to obesity and its complications in well-developed Western societies. Impairment of liver regeneration was observed after partial hepatectomy in mice fed a high-fat diet. Fatty livers were more susceptible to posthepatectomy damage and failure. The underlying molecular mechanism was associated with increased inhibitor of nuclear factor-kappa B alpha (IκBα) expression, which inhibited nuclear factor-kappa B (NF-κB) activation and induction of its target genes, cyclin D1 and Bcl-xL, increasing sensitivity to apoptosis initiated by elevated tumor necrosis factor-alpha. In addition, since mice fed with a high-fat diet have higher leptin levels caused by increased adiposity, our work supports the hypothesis that the impairment of regeneration previously seen in genetically obese mice indeed results from liver steatosis rather than the disruption of leptin signaling. In conclusion, high fat in the diet impairs liver regeneration and predisposes steatotic livers to increased injury through IκBα overexpression and subsequent NF-κB inhibition. (HEPATOLOGY 2005;42:1148-1157.)

See Editorial on Page 1001

Obesity is becoming an increasing health problem in modern Western societies, with approximately 30% of the adult population of the United States being clinically obese.1 This is often a consequence of bad dietary habits (high amounts of fat and carbohydrate in the diet) and low physical activity.2 Although overweight individuals have been shown to be at risk for the development of serious complications such as ischemic heart disease, arterial hypertension, and diabetes type II,2 relatively little is known about the impact of diet and obesity on liver pathophysiology.

The accumulation of triglycerides in liver parenchymal cells (fatty change, or steatosis), a common liver pathology, is a well-established effect of obesity.3 The importance of liver steatosis, however, may be seriously underrated, since it is commonly considered a benign condition without significant clinical consequences. The surgical removal of part of the liver (partial hepatectomy [PHx]) is usually a well-tolerated procedure, in part because the liver has an enormous functional reserve and unique regenerative capabilities.4 However, pre-existing pathological abnormalities such as liver steatosis may significantly deteriorate the postoperative course after surgical resection.5-7

In many clinical and experimental situations, delayed postoperative recovery of the steatotic liver has been related to defective cell proliferation and associated with

Abbreviations: PHx, partial hepatectomy; NF-κB, nuclear factor-kappa B; HFD, high-fat diet; IκBα, inhibitor of nuclear factor-kappa B alpha; BrdU, 5-bromo-2’-deoxyuridine; RT-PCR, reverse transcriptase-polymerase chain reaction; EMSA, electrophoretic mobility shift assay; STAT3, signal transducer and activator of transcription 3; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6.

From the Departments of1 Pathology and Laboratory Medicine,2 Genetics, and 3 Surgery, University of Pennsylvania Medical School, Philadelphia, PA.

Received October 27, 2004; accepted July 26, 2005.

Supported in part by NIH grants DK49629 (R.T.) and DK59422 (J.D.L.). The authors thank the Digestive and Liver Center grant P30 DK50306 for technical support.

R.T. is currently affiliated with the Department of Metabolic Diseases, Hoffmann-La Roche, Nutley, NJ.

Address reprint requests to: Dr. John D. Lambris, University of Pennsylvania, 401C Stellar Chance, 422 Curie Blvd., Philadelphia, PA 19104. E-mail: lambris@mail.med.upenn.edu; fax: 215-573-8738.

Copyright © 2005 by the American Association for the Study of Liver Diseases. Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hep.20879

Potential conflict of interest: Nothing to report.
increased liver damage. Nuclear factor-kappa B (NF-κB), crucial for the priming phase of liver regeneration, has been shown to be involved in both of these processes, promoting hepatocyte proliferation and also inhibiting apoptosis of liver cells.  

To study the effects of dietary-induced obesity on liver regeneration, we subjected mice fed a high-fat diet (HFD) to PHx. We found that sustained feeding of an HFD resulted in liver steatosis and considerably impaired regeneration after PHx. The defect in liver regeneration was associated with higher animal morbidity, the development of transient liver failure, and increased liver apoptosis and was related to inhibition of the NF-κB pathway resulting from increased cytoplasmic concentrations of inhibitor of NF-κB alpha (IκBa).  

Our data indicate that an HFD and resulting liver steatosis impairs liver regeneration after surgical resection, and although liver fatty changes are considered to be a reversible and relatively benign pathology, under certain circumstances they may result in a predisposition to serious clinical complications.

Materials and Methods

Animal Studies. Starting at weaning age, male C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) had free access to either a standard diet containing 6.5% (wt/wt) fat (17.3% of calories), 47% (wt/wt) carbohydrates (5.47% sucrose; 55.1% of calories), and 23.5% (wt/wt) protein (27.6% of calories) (mouse chow 5008; Ralston-Purina Co., St. Louis, MO) or a custom diet containing 21% (wt/wt) anhydrous milk fat (40.8% of calories), 49.2% (wt/wt) carbohydrates (70% sucrose; 42.2% of calories), and 19.8% (wt/wt) protein (17.0% of calories) (Adjusted Calories Western Type Diet 88137; Harlan Teklad Premier Laboratory Diets, Madison, WI), referred to in the text as an HFD. Mice were used with approval of the University of Pennsylvania Institutional Animal Care and Use Committee and under National Institutes of Health guidelines.

For PHx studies, animals 12 to 16 weeks of age were anesthetized and subjected to midventral laparotomy with (PHx) or without (sham) 70% liver resection. At least three animals in each cohort were sacrificed at each time point analyzed. One hour prior to sacrifice at time points of 0 hours or more than 12 hours, a single dose of BrdU (5-bromo-2’-deoxyuridine; Sigma, St. Louis, MO) was injected intraperitoneally at a dose of 50 mg/kg animal weight. At the time of sacrifice, mice were anesthetized, blood was harvested from the right ventricle of the heart, and the remaining liver lobes were removed, weighed, and processed for protein, RNA, or histological analysis. Serum was analyzed (Anlytics Inc., Gaithersburg, MD) for albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose, total cholesterol, triglycerides, and total bilirubin.

Liver Histology and Immunohistochemical Stainings. Liver morphology was assessed based on 5-μm hematoxylin and eosin–stained paraffin sections. Hepatocyte nuclear staining for BrdU was performed essentially as described. Total BrdU-labeled hepatocytes were determined by counting positively stained hepatocyte nuclei in 10 high-power microscope fields (×400) per liver.

Periodic Acid Schiff’s/Diastase staining was performed to exclude glycogen deposition as a cause of microvesicular hepatocyte degeneration.

Apoptotic cells in the liver were detected through immunostaining with a monoclonal antibody recognizing the cleavage site of activated caspase-3 on cytokeratin-18, as previously described.

Extract Preparation. Nuclear and whole-cell protein extracts from the liver were prepared in the presence of protease and phosphatase inhibitors as previously described. Total liver RNA was prepared as described.

Reverse Transcriptase-Polymerase Chain Reactions. Reverse transcriptase-polymerase chain reaction (RT-PCR) studies were performed using a QIAGEN One-Step RT-PCR Kit (QIAGEN Inc., Valencia, CA) according to the manufacturer’s instructions, with 1.5 μg pooled template RNA per reaction. Primers were as follows: Bcl-xL sense, 5’-CAGCCAGCCACCACTCTCCTC-3’, Bcl-xL antisense, 5’-TGCGGCTCTAATGTCCTATCTTC-3’ (cDNA length 316 bp); cyclin D1 sense, 5’-GGCCGGATGAGAACAACGCAGACA-3’, cyclin D1 antisense, 5’-ACCAGCCTCTCTCCTCTCAG-3’, cyclin D1 antisense, 5’-GGCTGAGATAATAGTGATGGATA-3’, β-actin sense, 5’-AGGGTGATGATGGAATTGGG-3’, β-actin antisense, 5’-AGCCAGACGTATCTCCTCTTGC-3’ (cDNA length 841 bp). All reactions had initial incubations of 30 minutes at 50°C followed by 15 minutes at 95°C. Amplification conditions were as follows: Bcl-xL, 45 seconds at 94°C, 45 seconds at 55°C, 45 seconds at 72°C (40 cycles); cyclin D1, 30 seconds at 94°C, 30 seconds at 67°C, 1 minute at 72°C (36 cycles); β-actin, 30 seconds at 95°C, 30 seconds at 60°C, 30 seconds at 72°C (36 cycles). All reactions had a final step of 10 minutes at 72°C. Ten microliters of each reaction were analyzed on agarose gels. β-Actin was used to normalize loading.

Electrophoretic Mobility Shift Assays and Super-shift Assays. Binding reactions were performed using 5 to 10 μg of nuclear extract essentially as described. The following oligonucleotide probes were used: NF-κB, 5’-AGTTGAGGGACTTCCCAGGCAGC-3’ (Promega
Corp., Madison, WI); signal transducer and activator of transcription 3 (STAT3), 5′-GATCCCTCCAGCATTTCCCCGTAATCCTCCAG-3′ (from the SIF binding element in the c-fos promoter).23 Rabbit reticulocyte lysate containing NF-κB was used as a positive control for NF-κB binding assays. For NF-κB supershift studies, 1/100 μL of Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) anti-p65 (C-20, catalogue no. sc-372X), anti-p50 (C-19, catalogue no. sc-1190X) or, as a negative control, anti-STAT3 (H-190, catalogue no. sc-7179X) antibody was added to the extract prior to addition of the probe.

**Immunoblots.** Twenty micrograms nuclear extract or 100 μg whole-cell extract were used for all immunoblots.17 Membranes were incubated with rabbit anti-mouse IκBα (C-21) or p65 (C-20) polyclonal IgG (Santa Cruz Biotechnology, Inc.), or with anti–β-actin mouse monoclonal (Sigma) antibodies (whole-cell extracts only) at 4°C. Primary antibody binding was detected using horseradish peroxidase–conjugated antibodies (Zymed Laboratories, Inc., San Francisco, CA; Jackson ImmunoResearch Laboratories Inc., West Grove, PA) and chemiluminescence (Amersham Pharmacia Biotech Inc., Piscataway, NJ). β-Actin blots (whole-cell extracts) or Ponceau S–stained membranes (nuclear extracts) were used to normalize protein loading.19 Rabbit reticulocyte lysate was used as a positive control for nuclear extract immunoblots.

**Assays for Tumor Necrosis Factor-Alpha Serum Concentrations.** Immunoassays for serum tumor necrosis factor-alpha (TNF-α) were performed using the OptEIA Mouse TNF-α Set (Pharmingen/BD Biosciences, Franklin Lakes, NJ) or Bio-Plex multiplex bead-based assays (Bio-Rad Laboratories, Hercules, CA), according to the manufacturer-supplied instructions.

**Data Analysis.** Densitometric analysis was used to quantitate protein levels (Image-Quant Software, Molecular Dynamics). RT-PCR products were quantitated using Kodak 1D Image Analysis software (Eastman Kodak Co., Rochester, NY). Microsoft Excel (Microsoft Corp., Redmond, WA) and Student t test were used for all statistical analyses performed. Statistical analyses were performed using at least 3 mice per time point. Error bars on graphs represent standard error from the mean. A Student t test result (P value) of .05 or less was considered to indicate a significant difference between groups.

**Results**

**An HFD Induces the Development of Increased Body Weight, Serum Metabolic Abnormalities, and Liver Steatosis in C57BL/6 Mice.** C57BL/6 mice fed a diet containing 21% fat (HFD) for 9 weeks gained more weight, on average, than did pair-fed control mice receiving a standard diet (6.5% fat content) (Fig. 1A). HFD-fed mice were 18% heavier than their control counterparts. This higher average body weight was associated with significantly elevated serum total cholesterol levels (Fig. 1B), although total triglyceride and glucose levels were similar in both groups (unpublished data). At the time of PHx, livers from HFD-fed mice revealed macroscopic and microscopic evidence of fatty changes. Gross examination showed the livers to be larger and paler than control livers. This observation was confirmed by a higher liver-to-body weight ratio in HFD-fed mice (Fig. 1C).

**HFD-Fed Mice Display Enhanced Liver Steatosis and Impaired Regeneration Following PHx.** Hematoxylin & eosin–stained liver sections harvested at various time points after PHx from HFD-fed mice revealed significantly increased steatosis when compared to prehepa-
tectomy specimens (Fig. 2A-B). Although fatty changes also appeared in livers of mice fed a regular diet after PHx (Fig. 2B), the extent of steatosis was considerably lower and similar to that normally observed.24 None of the sham-operated mice showed an increase in liver steatosis after surgery (unpublished data).

Mice fed an HFD displayed impaired liver regeneration following PHx, as illustrated by a slower restoration of liver mass (Fig. 3A) and a lower number of BrdU-positive hepatocyte nuclei (Fig. 3B) when compared to controls. Hepatocyte DNA synthesis reached its maximum at 40 hours after PHx in both groups of animals. However, the mean value of BrdU incorporation was 85% lower in steatotic livers than in controls (Fig. 3B-C). Lower cell proliferation was associated with a decrease in the induction of RNA for cyclin D1, which is an essential factor for cell cycle re-entry (Fig. 3D-E).25

PHx Causes Transient Liver Failure and Liver Injury in Mice Fed an HFD. Although mortality rates were similar in both cohorts (~15%), 26% of HFD-fed mice showed manifestations of clinical deterioration after PHx not related to surgical complications, whereas none of the control mice exhibited these symptoms. HFD-fed mice had significantly higher basal levels of aminotransferases in serum, though values were still within the normal range (Fig. 4A-B). At 8 hours after PHx and later in these mice, significantly higher serum total bilirubin lev-

---

Fig. 2. Feeding mice a high-fat diet results in liver steatosis. (A) Hematoxylin and eosin staining of 0-hour prehepatectomy livers from mice fed a diet containing 6.5% or 21% fat. (B) Livers at 40 hours after partial hepatectomy. Magnification, ×200.

Fig. 3. Mice fed a high-fat diet (HFD) have impaired liver regeneration after partial hepatectomy (PHx). Graphs representing (A) restoration of liver mass and (B) counts of BrdU-positive hepatocyte nuclei at various time points after PHx in control (6.5% fat) and HFD-fed (21% fat) mice. (C) BrdU staining of livers from control and HFD-fed mice 40 hours after PHx. Magnification, ×400. (D) RT-PCR for cyclin D1 using pooled samples of liver RNA from control and HFD-fed mice. (E) Graph representing quantitation of cyclin D1 RT-PCR. In panels A, B, and E, black squares indicate control mice; white circles, HFD-fed mice. *P < .05; **P < .01. BrdU, 5-bromo-2′-deoxyuridine; HPF, high-power field.
els, indicating transient liver failure, and increased serum aminotransferases, suggesting augmented injury, were seen when compared to control littermates (Fig. 4). Bilirubin and aminotransferase levels did not return to baseline until 60 hours after the procedure in HFD-fed mice, whereas normalization occurred in control mice much earlier (Fig. 4). Despite existing differences in bilirubin levels, we did not observe hypoalbuminemia in mice fed an HFD (unpublished data).

**Acute Liver Failure Correlates With a High Rate of Apoptotic Cell Death and Impaired Antiapoptotic Signaling in Steatotic Livers.** To determine the mode of liver tissue injury in HFD-fed mice, we examined the presence of apoptotic liver cells. Up to 8 hours, a low and similar amount of apoptosis (grades 1-2) was seen in both control and steatotic livers (Fig. 5A-B; see legend for grading explanation). From 8 hours onward, very little apoptosis was seen in control livers (grade 0), whereas steatotic livers displayed a significantly increasing rate of cell death (grades 1-4), peaking at 36 hours. This apoptosis was nearly resolved by 48 hours and no longer detectable by 60 hours, correlating with the pattern of serum aminotransaminasemia observed in these mice.

Increased apoptosis in steatotic livers was correlated with lower induction of the antiapoptotic factor Bcl-xL. Agreeing with previous studies, we observed a rapid increase in Bcl-xL RNA levels following PHx in control livers (Fig. 5C-D). Steatotic livers had slightly higher starting levels of Bcl-xL RNA (1.18 times higher, unpublished data), but had an overall decrease in Bcl-xL expression after PHx, with levels remaining at or below baseline at all time points.

**Post-PHx NF-κB Activity Is Reduced in Livers of Mice Fed an HFD.** The phenotype and altered gene induction for cyclin D1 and Bcl-xL observed in HFD-fed mice may have been the result of disturbances in the regulatory mechanisms that affect cellular proliferation and survival, in which TNF-α–regulated NF-κB is essential. Supershift EMSA experiments showed that both p50/p65 heterodimers and p50 homodimers of NF-κB were present in livers from control as well as HFD-fed mice (unpublished data). In both groups, an early peak of NF-κB DNA-binding activity was seen 30 minutes to 1 hour after PHx (Fig. 6A). However, the level of induction of transcriptionally active p50/p65 heterodimers was significantly lower in steatotic than in control liver extracts at 30 minutes (P < .05, Fig. 6B). Furthermore, at 8 hours after PHx, there was a second increase in activity occurring only in the control livers (Fig. 6A-B). Thus, steatotic livers displayed decreased NF-κB activity at 30 minutes and 8 hours after PHx.

**NF-κB Nuclear Translocation Is Impaired After PHx in Livers of HFD-Fed Mice.** The nuclear expression of p65 was significantly lower at 1 and 8 hours after surgery in HFD-fed mice when compared to control livers, as shown by immunoblot analysis (Fig. 6C-D; P < .05). However, the baseline levels of p65 expression in both whole-cell and nuclear extracts were similar (Fig. 6E). Moreover, we saw no changes in the expression of p65 in whole-cell extracts at any of the assessed time points after PHx in either cohort (unpublished data), indicating that decreased NF-κB binding activity was caused by impaired NF-κB translocation into the nucleus. In addition, the kinetics of changes in nuclear p65 expression (Fig. 6C-D) followed a pattern similar to that seen for DNA binding activity.

**IkBα Expression is Higher in Steatotic Livers Than in Control Livers.** The decrease in DNA binding activity and impairment of NF-κB nuclear translocation observed in livers from HFD-fed mice suggested that NF-κB was being sequestered in the cell cytoplasm. This sequestration was likely caused by IkBα, which binds to NF-κB in the cytoplasm of the cell and prevents it from
entering the nucleus and binding to DNA, until activation signals cause its degradation. Indeed, immunoblot analysis of whole-cell extracts showed nearly twice as much IκBα in quiescent steatotic livers as in control livers (P < .05, Fig. 7). Following PHx in control livers, there was a decrease in IκBα expression from 30 minutes to 2 hours, the time of peak NF-κB activation. The degradation of IκBα was not functionally involved in the second induction of NF-κB activation, which occurred 8 hours after PHx only in control livers; by this time, the amount of IκBα had returned to quiescent levels. In steatotic livers, there was a decrease in the amount of IκBα immediately after PHx, with levels averaging lower than baseline at all subsequent time points (Fig. 7). However, out to 2 hours after PHx, the levels of IκBα expression remained higher in steatotic livers than in controls (P < .05 at 1 hour).

**HFD-Fed Mice Do Not Display Impairment of the Interleukin-6 Signaling Pathway Despite Higher Induction of TNF-α Following PHx.** NF-κB, regulated by TNF-α,11,15 partially regulates interleukin-6 (IL-6),28 which induces STAT3 during liver regeneration.21 Since NF-κB activity was decreased in post-PHx livers from mice fed an HFD, the activities of TNF-α and IL-6 signaling were analyzed. Immunoassays showed a trend for higher TNF-α levels and induction in serum at very early time points after PHx in HFD-fed mice, with statistically significant differences at 1 hour, when compared to controls (Fig. 8A). However, higher TNF-α induction did not influence IL-6/STAT3 signaling, as shown by EMSA.
analysis of STAT3 DNA binding (Fig. 8B). Thus, NF-κB inhibition observed in mice fed an HFD did not result from lower TNF-α induction, and the phenotype of
HFD-fed mice was not a result of disruption of the IL-6/STAT3 pathway.

Discussion

The clinical significance of liver steatosis depends upon the extent of lipid accumulation in liver parenchymal cells. However, in light of current knowledge it is almost impossible to draw a line separating benign steatosis from clinically relevant cellular degeneration, especially when considering the wide variety of pathophysiological situations in which the liver may be involved. In our study, mice fed an HFD developed transient acute liver failure after PHx. The inability of the liver to maintain its metabolic functions was mainly the result of severely impaired liver regeneration and an increased rate of apoptotic cell death. The elevated levels of aminotransferases in HFD-fed mice after PHx along with increased apoptosis indicated a vulnerability of steatotic livers to surgical insult. It has been shown that hepatocyte steatosis predisposes livers to the development of necrosis and inflammatory changes after transplantation. Liver injury under these circumstances is likely induced by LPS released from the gut. PHx, used here as an experimental model, is also associated with an increased concentration of gut-derived LPS in the portal blood. The activation of TNF-α as a consequence of LPS stimulation may contribute to the higher levels of apoptosis seen in steatotic livers. Moreover, it has been shown that due to increased adiposity, mice fed an HFD have elevated levels of leptin, which, besides the regulation of food intake, also has immunomodulatory functions. In particular, leptin augments inflammation through an increased release of cytokines, such as TNF-α, which is released in large quantities from tissue macrophages, including Kupffer cells in the liver. Indeed, our results suggest higher TNF-α induction after PHx in HFD-fed mice.

It appears that the increased vulnerability of steatotic livers, along with their inability to regenerate after PHx, is primarily caused by impaired NF-κB activation, since this transcription factor is essential for liver cell proliferation and survival. The primary defect observed in steatotic liver cells includes overexpression of the NF-κB inhibitor IκBα. Since IκBα degradation is required for NF-κB pathway activation, constitutively increased cytoplasmic quantities in steatotic liver cells (even before PHx) prevents activation of NF-κB. However, the reason for IκBα overexpression remains unclear. We can speculate that various metabolic abnormalities observed in steatotic hepatocytes, including increased lipid peroxidation along with impaired mitochondrial oxidation and a subsequent deficit in adenosine triphosphate (a major source of cell energy), might participate in inadequate degradation of IκBα in steatotic liver cells.

Our current data suggest the following model for impaired liver regeneration in HFD-fed mice: LPS-stimulated TNF-α levels are increased after PHx, possibly due to amplified leptin signaling. However, high levels of IκBα prevent TNF-α from activating NF-κB. As a result, the induction of some NF-κB targets, such as the cell signaling molecule cyclin D1 and hepatoprotective factor Bcl-xL, but not the IL-6/STAT3 pathway, is impaired. The end result is decreased hepatocyte proliferation and increased apoptotic liver cell death, ultimately impeding liver regeneration in these mice.

Despite lower induction of cyclin D1 RNA following PHx, HFD-fed mice had approximately 1.6 times more cyclin D1 RNA than controls in quiescent livers (unpublished data). However, no increases in BrdU incorporation were observed in these 0-hour livers, indicating that higher cyclin D1 RNA in resting steatotic livers may be an adaptive state, and that an increase in basal gene expression is required for entry into the cell cycle.

Notwithstanding increased susceptibility to damage and impaired regeneration, some mice fed an HFD were able to eventually recover from PHx, with serum aminotransferase and bilirubin levels returning to baseline values by 48 to 60 hours. The kinetics of delayed recovery correlated with the severity of apoptotic changes in the liver. The aminotransferase levels returned to normal values as a consequence of the resolution of apoptosis, seen in liver sections by 48 hours after PHx.

The impairment of liver regeneration and NF-κB activation observed in our study has also been seen in genetic models of obesity (ob/ob mice and fa/fa rats) following PHx. However, there is an interesting difference in leptin levels between HFD-fed models and genetic models of obesity. As previously mentioned, mice fed an HFD have increased leptin levels, due to an increased amount of adipose tissue. It has been suggested that impairment of liver regeneration in ob/ob mice and fa/fa rats may be caused by the disruption of leptin signaling rather than the accumulation of triglycerides in liver parenchymal cells. Our results suggest that the defect in regeneration observed in genetically obese mice depends on an increased accumulation of triglycerides in liver parenchymal cells and is not a consequence of leptin deficiency. However, potential adaptive changes to leptin deficiency in genetically obese rodents leave open the possibility of a direct impairment of liver regeneration by downstream leptin signals.

In contrast to our results, some other studies of dietary-induced steatosis have not revealed any differences in regeneration between fatty and control livers. Some of these
studies, such as those using a methionine-choline deficient diet, are not directly comparable to our own since they involve an element of liver inflammation.42,43 Also, the lack of impaired liver regeneration after feeding rats a methionine-choline deficient diet may be caused by choline deficiency, as this has been shown to enhance regeneration in rat livers,44 while increased choline can inhibit this process.45 In other work using a diet supplemented with orotic acid, there was no observable increase in the liver-to-body weight ratio.43 Livers from these rats showed only microvesicular steatosis, which alone has not been shown to affect regenerative abilities of the liver.46 The lack of both macrovesicular liver steatosis and an increase in the liver-to-body weight ratio, as observed in HFD-fed mice, suggests that the severity of hepatic steatosis was comparatively less in orotic acid–fed rats.

It should be noted that hyperinsulinemia and insulin resistance have been observed in some models of HFD-induced obesity.47,48 This discrepancy may be related to the total percentage of calories originating from fat in the diet. Significant abnormalities in glucose metabolism were observed in experimental mice when up to 80% of the calories delivered originated from fat.47,48 The energetic demands of mice used in our study were covered by significantly fewer (41%) calories from fat, and glucose metabolism was not significantly affected. Consequently, the lack of insulin resistance in our model may explain discrepancies with other studies using HFD-induced obesity, where increased NF-κB activity was observed in the liver and shown to be responsible for decreased insulin sensitivity.49 In addition, carbohydrates, including sucrose, constitute an important source of calories in the HFD used for our experiments. Although the sucrose content was significantly lower in the control diet (5.47% vs. 70% of total carbohydrate calories), we do not anticipate that this carbohydrate contributed to the observed defect in liver regeneration. Results published previously have indicated that an increased carbohydrate content does not have detrimental effects on the regenerative response after PHx.50

In conclusion, although it has previously been shown using genetic models of obesity that liver steatosis impairs regeneration after PHx, our studies clearly indicate that this defect occurs in a clinically relevant model of dietary-induced obesity. We have determined that the primary defect leading to impaired regeneration and the vulnerability of steatotic livers to injury after surgical resection is associated with constitutively occurring overexpression of IkBα. This abnormality, through the inhibition of NF-κB activation and its targets, leads to an impaired hyperplastic response of liver cells and massive apoptosis. Moreover, high leptin levels, associated with the increased adiposity of mice fed an HFD, might aggravate the inflammatory activity of TNF-α, which is known to induce liver injury in various experimental models.

Acknowledgment: We thank Dr. D. McClellan for editorial assistance. We acknowledge the technical support of the Morphology Core of the Penn Center for Molecular Studies in Digestive and Liver Disease.

References

18. Markiewski MM, Mastellos D, Tudoran R, DeAngelis RA, Strey CW, Franchini S, et al. C3a and C3b activation products of the third compo-
41. Farrell GC, Robertson GR, Leclercq I, Horsmans Y. Liver regeneration in obese mice with fatty livers: does the impairment have relevance for other types of fatty liver disease? HEPATOLOGY 2002;35:731-732.