TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Leptin attenuates ischemia–reperfusion injury in the rat liver

Marco Carbone,[1] Luisa Campagnolo,[2] Mario Angelico,[1] Giuseppe Tisone,[3] Cristiana Almerighi,[1] Claudia Telesca,[1] Ilaria Lenci,[1] Ilana Moscatelli,[2] Renato Massoud[4] and Leonardo Baiocchi[1]

1 Hepatology Unit, Department of Internal Medicine, University of Tor Vergata, Rome, Italy
2 Histopathology Unit, University of Tor Vergata, Rome, Italy
3 Transplant Surgery Unit, University of Tor Vergata, Rome, Italy
4 Pathology Unit, University of Tor Vergata, Rome, Italy

Keywords
ischemia–reperfusion injury, leptin, liver, rat, STAT3.

Correspondence
Leonardo Baiocchi MD, PhD, Assistant Professor in Gastroenterology, Hepatology Unit, Department of Internal Medicine, University of Tor Vergata, Via Montpellier 1 – 00133 Rome, Italy.
Tel.: +39(6) 72596875;
fax: +39(6) 72596875;
e-mail: baiocchi@uniroma2.it

Conflict of Interest
None.

Received: 13 March 2012
Revision requested: 21 April 2012
Accepted: 8 August 2012
Published online: 13 September 2012

doi:10.1111/j.1432-2277.2012.01555.x

Summary

Leptin is an adipocytokine that reduces ischemic damage in several organs including brain and heart. STAT3 activation is a key step for the attainment of leptin effects in various tissues. We evaluated the possible effect of leptin on liver viability and STAT3 activation, in a rat model of ischemia–reperfusion injury. Rat livers, flushed and stored with Belzer solution (4° C for 24 h), were warmly reperfused (3.5 ml/min/g liver for 1 h at 37° C with O_2) with Krebs–Ringer bicarbonate. Treatment group underwent an identical protocol with the adjunct of Leptin (10 ng/ml). Liver effluent was harvested to assess LDH and AST output. Liver tissue was used for pSTAT3 expression (western blot and immunostaining), optical microscopy, TUNEL, and Cell Death Detection assays. The pSTAT3 expression was enhanced by administration of leptin. In parallel, LDH and AST output were reduced ($P = 0.04$ and $P = 0.02$ for LDH and AST, respectively). Optical microscopy, TUNEL, and Cell Death Detection assay results demonstrated increased viability in livers treated with leptin in comparison with others (Optical microscopy $P = 0.02$; TUNEL $P = 0.01$; Cell death Detection assay $P = 0.003$). In conclusion, cold storage and reperfusion with leptin reduce liver ischemia–reperfusion injury. This effect is associated with an increased expression of pSTAT-3.

Introduction

Ischemia–reperfusion injury (IRI) is responsible for significant organ dysfunction and failure after liver transplantation (LT), liver resection, and hemorrhagic shock [1–3]. In the setting of LT, the major damages are determined during graft cold preservation and subsequent warm reperfusion at implantation. Necrosis and/or apoptosis are the predominant mode of cell death following this procedure [1].

The complex mechanisms involved in liver IRI are far from being completely elucidated and possible factors contributing to damage include cellular and humoral processes, endogenous signal cascades, and others. Cold storage appears to cause injury to sinusoidal endothelial cells (SECs), whereas warm reperfusion affects both hepatocytes and endothelial cells.

Several laboratories reported evidence for apoptotic cell death during hepatic ischemia–reperfusion [4,5]. According to these studies, nearly 50% of both endothelial cells and hepatocytes undergo apoptosis during reperfusion.

Modulation of Signal Transducer and Activator of Transcription-3 (STAT-3) has been considered a possible strategy to reduce IRI damage in several organs including liver [6–8], heart [9,10], and intestine [11]. Under appropriate stimuli, STAT-3 undergoes phosphorylation and translocation in the nucleus where it enhances expression of survival and antiapoptotic genes [12–18]. In this

perspective, any possible factor increasing STAT-3 activity may be interesting as a therapy against liver IRI.

Leptin, a circulating hormone secreted by adipocytes, influences body weight homeostasis through effects on food intake and energy expenditure [19]. Binding of leptin to its receptor long-form determines activation of Janus tyrosine kinase 2 (JAK-2) and in turn phosphorylation and activation of target proteins including STAT-3. On the other hand, STAT-3 activation is considered a key step for different leptin biological effects as demonstrated in experimental models in which this process was suppressed by a specific phosphopeptide STAT-3 inhibitor [20].

Given the leptin-related activation of STAT-3 and the beneficial effect of the latter on IRI, several authors have evaluated the role of this hormone in attenuating ischemic damage in heart [21], brain [22,23], intestine [24,25], and kidney [26] with encouraging results. Possible effects of leptin on liver IRI have so far never been assessed. The aim of this study was to evaluate the effect of leptin administration on an isolated perfused rat liver model of IRI.

Materials and methods

Animal model

Surgical procedure and cold ischemia

All procedures were in compliance with the standard indications for animal care and treatment of our institution deriving from NIH Guide for the Care and Use of Laboratory Animals. Male Wistar rats were obtained from Charles River (Milan, Italy). Animals were allowed free access to rodent food and had a 12-h day–night rhythm before experiments. Normal control rats ($n = 8$; 200 ± 10 g body weight) were sacrificed shortly after anesthesia (sodium pentobarbital 50 mg/kg body weight, intraperitoneally), the others underwent the following protocol of IRI and treatment as previously described [27]. The abdomen was incised and the pancreatoduodenal collateral branch of portal vein ligated. After systemic heparinization, the portal vein and the inferior vena cava were cannulated with an 18-gauge and a 16-gauge Teflon intravenous catheter, respectively. Livers were flushed and stored at +4° C with 50 cc of Belzer solution (control $n = 10$; 200 ± 10 g body weight), or Belzer + Leptin 10 ng/ml (treated $n = 10$; 200 ± 10 g body weight). Livers were then stored for 24 h at +4° C in Belzer solution (cold ischemia time).

Warm reperfusion

Livers were reperfused for 1 h with Krebs–Ringer bicarbonate (KRB) solution (NaCl 118 mM, KCl 4.8 mM, $NaHCO_3$ 25 mM, KH_2PO_4 12mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 1.9mM, D-glucose 5.5 mM) at 37° C, pH 7.4, gassed with O_2-Co_2 (95–5%) at 3.5 ml/min/g liver. Leptin was added to the perfusion media, at a physiological concentration of 10 ng/ml as previously described by others [28,29]. Grafts were transferred into a temperature-controlled chamber. Aliquots of effluent were harvested every 5 min to assess lactate dehydrogenase (LDH) release. At the end of the experiments, tissue specimens were formalin-fixed and paraffin-embedded, or snap frozen in liquid nitrogen or homogenized before freezing at −80° C, for further analysis.

Biochemical analysis

Lactate dehydrogenase and AST were determined spectophotometrically using a Beckman DU-640 apparatus (Fullerton, CA, USA) employing commercially available kits (Bio-Sud Spa, Riardo, Italy) and following the instructions of the vendor, as previously described [27].

Immunoblot analysis

STAT-3 and pSTAT-3 western blot analysis were conducted as previously described [27,30]. Whole liver tissue specimens were homogenized in RIPA buffer (50 mM Tris pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% deoxycholic acid, 0.1% sodium dodecyl sulfate, 2 mM ethylediaminetetracetic acid, 10 mM NaF, 10 μg/ml Leupeptin, 20 μg/ml aprotinin, imM phenylmethylsulphonil fluoride). Proteins (50 μg) were then resolved on a sodium dodecyl sulfate 7.5% polyacrylamide gel electrophoresis and blotted on a nitrocellulose membrane (Bio-Rad, Hercules, CA, USA). The membrane was blocked with a 5% solution of nonfat dry milk in TBST (50 mM Tris, 150 mM NaCl with 0.05% Tween 20). After overnight incubation (+4° C) with STAT-3 or phosphorylated STAT-3 (pSTAT-3) primary antibody in TBST, membranes were washed five times in TBST and incubated for 1 h (room temperature) with secondary antibody. Excess antibody was removed by washing five times with TBST and proteins were visualized using chemiluminescence (ECL Plus Kit; Amersham Life Science, Milan, Italy). The intensity of bands was evaluated by scanning video densitometry employing a Chemilmager 4000 low light imaging system (Alpha Innotech Corp, San Leandro, CA, USA).

Immunostaining

Frozen sections (5 μm) from three separate experiments were fixed in absolute methanol (30 min at −20° C), and washed three times in phosphate-buffered saline (PBS) (pH 7.4). Sections were then blocked with 10% serum, 0.5% bovine serum albumin, and 0.5% of Triton X-100 in PBS for 30 min. Slides were washed again with PBS three times for 5 min and incubated overnight with primary antibody. After washing with PBS, slides were incubated

with a biotin-labeled secondary antibody (Dako Italia, Milan-Italy) for 1 h at room temperature. A peroxidase-labeled avidin–biotin complex with diaminobenzidine was used as a substrate for the reaction. Sections were washed again with PBS, a coverslip was mounted, and sections were examined under the microscope (BX 40; Olympus Optical Co., Milan, Italy). The number of p-STAT-3 positive cells per field was evaluated under a 20× objective. Ten fields for each separate experiment were examined.

Optical microscopy and terminal deoxynucleotidyl transferase dUTP Nick End Labeling (TUNEL) assay for assessment of apoptosis

The number of apoptotic cells was assessed in formalin-fixed paraffin-embedded liver tissue specimens. For optical microscopy, ematoxilin–eosin-stained liver sections were examined under a 20× objective and apoptosis was identified according to the presence of chromatin condensation, cell shrinkage, and apoptotic bodies. Ten fields for each separate experiment were examined and results were expressed as apoptotic nuclei per field. For TUNEL assay, we employed the *In situ* Cell Death Detection Kit (Roche, Milan, Italy) according to the instructions of the vendor. Slides were analyzed under a fluorescence microscope (BRC 600 laser scanning confocal microscope) using as positive controls, sections incubated with DNase I (3000 U/ml for 10 min at room temperature). The number of apoptotic nuclei was evaluated under a 20× objective and expressed as apoptotic nuclei per field. Ten fields for each separate experiment were examined.

Cell death detection assay

For detection of apoptotic nucleosomes, Cell Death detection ELISA assay (Roche, Milan, Italy) was employed according to the instructions of the vendor and as previously described by others [31]. The cytoplasmic fraction of frozen tissue lysates was analyzed using a Beckman DU-640 apparatus. Results were expressed as absorbance at 405 nm for μg of liver.

Reagents

All reagents were from Sigma-Aldrich (Milan, Italy), unless otherwise specified. Purified mouse anti-STAT3 and anti-pSTAT3 and secondary HRP-labeled antibody were from Becton-Dickinson (Milan, Italy).

Statistical analysis

Data were analyzed using the NCSS 2000 software package (NCSS, Kaysville, UT, USA). Differences between groups were evaluated with the Student's *t*-test for unpaired data or ANOVA for multiple comparison groups. A $P < 0.05$ was considered statistically significant.

Results

LDH and AST release during reperfusion

Sinusoidal efflux of LDH and AST (U/min × g liver) during warm reperfusion was studied in 5-min aliquots as a biochemical marker of cytolysis. Livers treated with IRI+leptin exhibited a reduced cumulative LDH and AST release in the perfusate in comparison with IRI livers (LDH 0.6 ± 0.4 vs. 0.3 ± 0.2, $P = 0.04$; AST 0.4 ± 0.2 vs 0.13 ± 0.07, $P = 0.02$). LDH and AST levels in control livers are also reported in Fig. 1 for comparison.

STAT-3 expression and activation

STAT3 and pSTAT-3 expression were assessed by immunoblot analysis (Fig. 2). These experiments demonstrated a uniform expression of STAT-3 comparing normal, IRI,

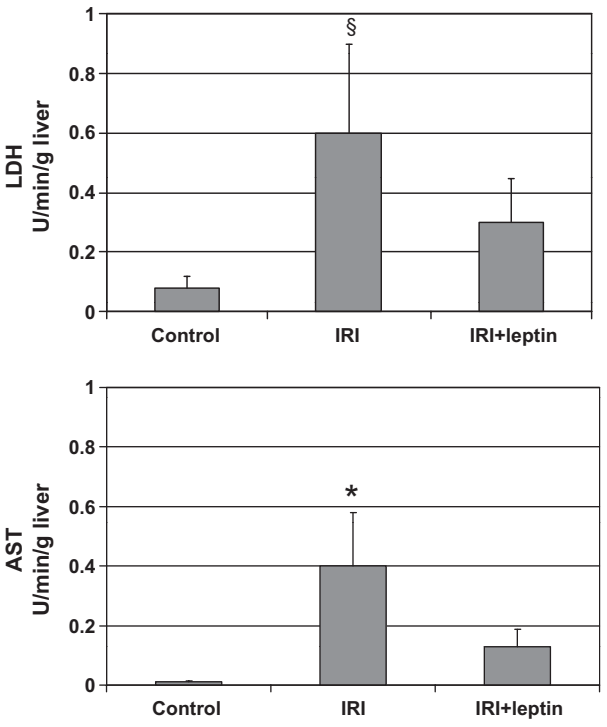

Figure 1 Graphic representation of cumulative output of LDH and AST in liver undergoing ischemia–reperfusion injury (IRI) and IRI+leptin. Data on normal liver are also reported for comparison. The addition of leptin determined a statistically significant decrease in liver enzymes output. §Statistically significant difference ($P = 0.04$) between IRI+Leptin and IRI. *Statistically significant difference ($P = 0.02$) between IRI+Leptin and IRI.

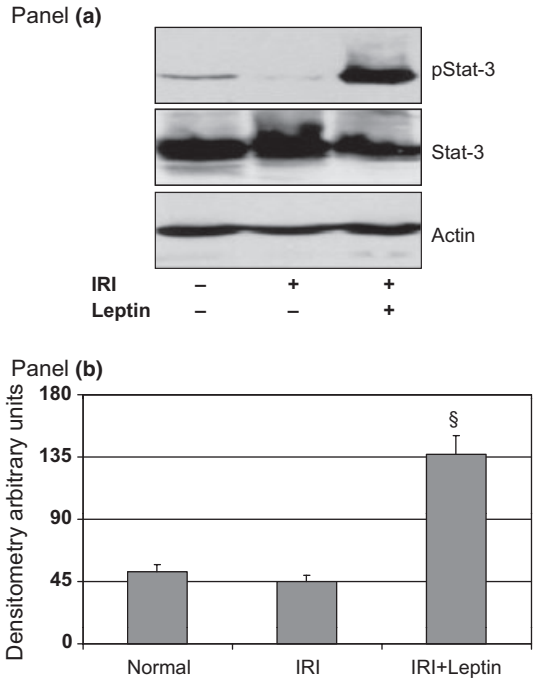

Figure 2 Panel a: Western blot analysis demonstrating increased expression of pSTAT3 in liver exposed to Leptin during ischemia–reperfusion injury (IRI). Normal liver served as control. In the same experiments, there were no differences in the expression of STAT3. Actin served as control. Panel b: Graphic representation of the different rate of expression of p-STAT3. §Statistically significant difference ($P = 0.001$) between IRI+Leptin and IRI.

or IRI+leptin livers. However, the expression of STAT-3 activated form p-STAT-3 was enhanced in livers undergoing IRI+leptin ($P = 0.001$). Enhanced expression of p-STAT-3 in IRI livers treated with leptin was also confirmed by immunostaining data. In fact, leptin-treated livers showed an increased number of p-STAT-3 positive cells in comparison with livers undergoing IRI only (IRI 3 ± 1.6 vs. IRI+Leptin 35.1 ± 4.4; $P < 0.001$).

Assessment of liver viability

Assessment of liver necrosis–apoptosis was carried out by optical microscopy examination, TUNEL, and Cell Death ELISA assays. Fig. 3 shows the comparative results obtained by optical microscopy and TUNEL assay in normal, IRI, and IRI+leptin livers. Leptin pretreatment determined a relevant reduction in nonviable cells in comparison with control IRI, that was evidenced by both optical microscopy and TUNEL assay ($P = 0.02$ and $P = 0.01$ for optical microscopy and TUNEL, respectively). These results were corroborated by the reduced levels of histone-containing DNA fragments in cytoplasmic fraction of IRI livers treated with leptin (Fig. 4).

Discussion

Different approaches are currently being investigated to minimize liver IRI. These may be divided into surgical or pharmacological strategies [32]. With regard to the latter, the use of antioxidant, vasoactive, or anti-inflammatory drugs has been tested with encouraging, but not conclusive results, also in humans.

Recently, adipocytokines, peptide hormones produced by adipose tissue, are gathering a growing interest because of their relationship with ischemic damage in cardiac

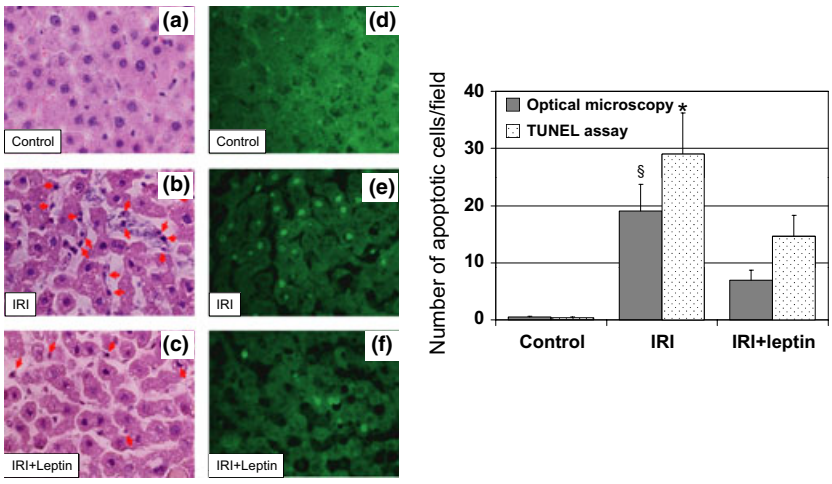

Figure 3 Optical microscopy (panel a, b, and c) and Terminal deoxynucleotidyl transferase dUTP Nick End Labeling (TUNEL) observations (panel d, e, and f) illustrate the net reduction in nonviable cells (red arrows for optical microscopy, nuclear staining for TUNEL) in liver undergoing ischemia–reperfusion injury (IRI) with the addition of Leptin in comparison with those exposed to IRI only. Normal liver served as control. The graph represents the number of positive cells x field (10 fields were evaluated for each separate experiment). §Statistically significant difference ($P = 0.02$) between IRI+Leptin and IRI. *Statistically significant difference ($P = 0.01$) between IRI+Leptin and IRI.

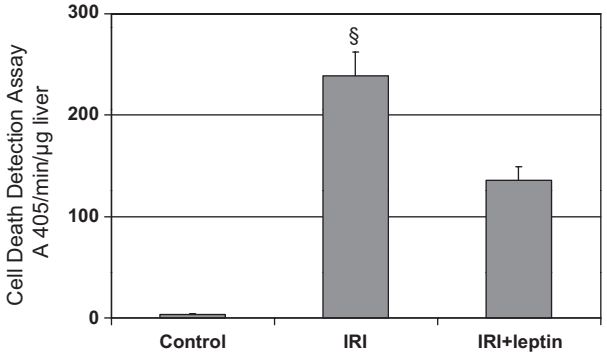

Figure 4 Cell Death Detection Assay results showing a statistically significant reduction in DNA fragmentation in liver undergoing ischemic–reperfusion injury (IRI) with the adjunct of leptin in comparison with those exposed to IRI only. Normal liver served as control. §Statistically significant difference ($P = 0.003$) between IRI+Leptin and IRI.

muscle [33]. Among them, leptin was evaluated as a possible cytoprotective and antiapoptotic hormone in various tissues exposed to ischemic damage. Different studies, conducted in various tissues such as brain [34], intestine [35], and heart [21], underscore the beneficial role of leptin administration in reducing ischemic cellular damage.

In regard to the liver, a few studies conducted on steatotic genetically leptin-deficient mice have supported an increased graft damage after IRI [36,37]. More recently, another study investigated fluctuations of endogenous leptin levels in a rat model of 70% liver IRI [38]. Although the author suggested a possible protective role of leptin in this setting, given the increased serum level of this hormone during ischemic damage, no data were shown to support this hypothesis.

With this background, we sought to evaluate, in a rat model of liver IRI, the possible effects of cold storage and reperfusion with leptin. Our results are the first demonstration that: (i) exposing the liver to leptin during IRI increases tissue viability; (ii) this phenomenon is associated with an enhanced expression of STAT3 in its phosphorylated–activated form.

The role of STAT3 activation in cellular protection and proliferation has been well documented. As regards the liver, IRI-induced apoptosis was demonstrated to be inhibited in primary hepatocyte cultures by STAT-3, probably with a mechanism involving manganese superoxide dismutase (Mn-SOD), a scavenger of reactive oxygen species [39]. However, other mechanisms may contribute to leptin cytoprotection during IRI, at least in cardiac muscle, such as modulation of mitochondrial potential changes and cytochrome C release [40].

Another interesting effect that might have an importance as regards the liver is the leptin-related protection from lipotoxicity [33]. The latter is a damage modality related to lipid overload in tissue as probably observed in non-alcoholic-steato-hepatitis (NASH) in humans and seems to be related to fatty acids-induced cell damage [41]. Lipotoxicity is characteristic of leptin deficiency status. In this perspective, it is possible to hypothesize that the use of leptin may protect steatotic marginal grafts from the widely reported increased IRI, thus allowing their use for LT in humans, a use for which they are usually excluded. However, this consideration remains speculative at this stage.

The possible contribution of oncotic necrosis or apoptosis in IRI of the liver is controversial [42–44]. Although some authors suggest oncotic necrosis to be the main modality of cell death during IRI of the liver [43], the complex process at the basis of the cellular insult suggests that these two death modalities may share a similar mechanism in this setting. In addition, liver damage during IRI is supported by two different phases: cold ischemia and warm reperfusion that may contribute differently to the onset of necrosis or apoptosis in the liver. Given the complexity of damage in this setting, and even though the net differentiation between cellular necrosis and apoptosis was behind the scope of this study, we decided to employ different and alternative methods to evaluate the viability of the liver.

First, we assessed cell necrosis monitoring LDH and AST levels during liver reperfusion, demonstrating that cold storage and reperfusion with leptin reduced IRI-induced liver enzyme release by one half. Then, we employed optical microscopy, TUNEL assay, and cell death ELISA assay. The first two methods were used as they are recognized as reliable strategies to indentify apoptotic bodies also in the liver, the third as it detects DNA fragmentation occurring both after apoptosis or oncotic necrosis. All tests demonstrated a nearly 50% improvement of tissue viability in the livers undergoing cold storage and reperfusion with leptin, allowing us to conclude that leptin attenuates liver damage induced by IRI, acting on apoptotic as well as on oncotic necrosis. In conclusion, our data support the findings that cold storage and reperfusion with leptin increase liver viability in a rat model of IRI. The possible extrapolation of these findings to the clinical fields of liver resection or transplantation in human is intriguing, but needs to be assessed.

Authorship

MC: writer, animal procedure, experiments. LC: experiments. MA: study design. GT: study design. CA: experiments. CT: experiments. IL: writer. IM: experiments. RM:experiments. LB: study design, writer, animal procedure.

Funding

Nothing to disclose.

Acknowledgements

The authors express gratitude to Alessandro De Stefano PhD for helping in the experiments and Serena Rotunno for revision of the English text.

References

1. Kim YI. Ischemia-reperfusion injury of the human liver during hepatic resection. *J Hepatobiliary Pancreat Surg* 2003; **10**: 195.
2. Rushing GD, Britt LD. Reperfusion injury after hemorrhage: a collective review. *Ann Surg* 2008; **247**: 929.
3. Abu-Amara M, Yang SY, Tapuria N, Fuller B, Davidson B, Seifalian A. Liver ischemia/reperfusion injury: processes in inflammatory networks – a review. *Liver Transpl* 2010; **16**: 1016.
4. Gao W, Bentley RC, Madden JF, Clavien PA. Apoptosis of sinusoidal endothelial cells is a critical mechanism of preservation injury in rat liver transplantation. *Hepatology* 1998; **27**: 1652.
5. Kohli V, Selzner M, Madden JF, Bentley RC, Clavien PA. Endothelial cell and hepatocyte deaths occur by apoptosis after ischemia-reperfusion injury in the rat liver. *Transplantation* 1999; **67**: 1099.
6. Matsumoto T, O'Malley K, Efron PA, *et al.* Interleukin-6 and STAT3 protect the liver from hepatic ischemia and reperfusion injury during ischemic preconditioning. *Surgery* 2006; **140**: 793.
7. He S, Atkinson C, Qiao F, Cianflone K, Chen X, Tomlinson S. A complement-dependent balance between hepatic ischemia/reperfusion injury and liver regeneration in mice. *J Clin Invest* 2009; **119**: 2304.
8. Caraceni P, Pertosa AM, Giannone F, *et al.* Antagonism of the cannabinoid CB-1 receptor protects rat liver against ischaemia-reperfusion injury complicated by endotoxaemia. *Gut* 2009; **58**: 1135.
9. Manukyan MC, Alvernaz CH, Poynter JA, *et al.* Interleukin-10 protects the ischemic heart from reperfusion injury via the STAT3 pathway. *Surgery* 2011; **150**: 231.
10. Barry SP, Townsend PA, McCormick J, *et al.* STAT3 deletion sensitizes cells to oxidative stress. *Biochem Biophys Res Commun* 2009; **385**: 324.
11. Jin X, Zimmers TA, Zhang Z, Pierce RH, Koniaris LG. Interleukin-6 is an important in vivo inhibitor of intestinal epithelial cell death in mice. *Gut* 2010; **59**: 186.
12. Shen Y, Devgan G, Darnell Jr JE, Bromberg JF. Constitutively activated Stat3 protects fibroblasts from serum withdrawal and UV-induced apoptosis and antagonizes the proapoptotic effects of activated Stat1. *Proc Natl Acad Sci USA* 2001; **98**: 1543.
13. Haga S, Terui K, Zhang HQ, *et al.* Stat3 protects against Fas-induced liver injury by redox-dependent and -independent mechanisms. *J Clin Invest* 2003; **112**: 989.
14. Barry SP, Townsend PA, Latchman DS, Stephanou A. Role of the JAK-STAT pathway in myocardial injury. *Trends Mol Med* 2007; **13**: 82.
15. Barry SP, Townsend PA, Knight RA, Scarabelli TM, Latchman DS, Stephanou A. STAT3 modulates the DNA damage response pathway. *Int J Exp Pathol* 2010; **91**: 506.
16. Briscoe CP, Hanif S, Arch JR, Tadayyon M. Leptin receptor long-form signalling in a human liver cell line. *Cytokine* 2001; **14**: 225.
17. Fava G, Alpini G, Rychlicki C, *et al.* Leptin enhances cholangiocarcinoma cell growth. *Cancer Res* 2008; **68**: 6752.
18. Saxena NK, Sharma D, Ding X, *et al.* Concomitant activation of the JAK/STAT, PI3K/AKT, and ERK signaling is involved in leptin-mediated promotion of invasion and migration of hepatocellular carcinoma cells. *Cancer Res* 2007; **67**: 2497.
19. Morris DL, Rui L. Recent advances in understanding leptin signaling and leptin resistance. *Am J Physiol Endocrinol Metab* 2009; **297**: E1247.
20. Buettner C, Pocai A, Muse ED, Etgen AM, Myers Jr MG, Rossetti L. Critical role of STAT3 in leptin's metabolic actions. *Cell Metab* 2006; **4**: 49.
21. Smith CC, Dixon RA, Wynne AM, *et al.* Leptin-induced cardioprotection involves JAK/STAT signaling that may be linked to the mitochondrial permeability transition pore. *Am J Physiol Heart Circ Physiol* 2010; **299**: H1265.
22. Zhang F, Wang S, Signore AP, Chen J. Neuroprotective effects of leptin against ischemic injury induced by oxygen-glucose deprivation and transient cerebral ischemia. *Stroke* 2007; **38**: 2329.
23. Zhang F, Chen J. Leptin protects hippocampal CA1 neurons against ischemic injury. *J Neurochem* 2008; **107**: 578.
24. Sukhotnik I, Helou H, Lurie M, *et al.* The effect of leptin on intestinal recovery following ischemia-reperfusion injury in a rat. *Pediatr Surg Int* 2007; **23**: 473.
25. Hacioglu A, Algin C, Pasaoglu O, Pasaoglu E, Kanbak G. Protective effect of leptin against ischemia-reperfusion injury in the rat small intestine. *BMC Gastroenterol* 2005; **5**: 37.
26. Erkasap S, Erkasap N, Koken T, *et al.* Effect of leptin on renal ischemia-reperfusion damage in rats. *J Physiol Biochem* 2004; **60**: 79.
27. Baiocchi L, Tisone G, Russo MA, *et al.* TUDCA prevents cholestasis and canalicular damage induced by ischemia-reperfusion injury in the rat, modulating PKCalpha-ezrin pathway. *Transpl Int* 2008; **21**: 792.
28. Nemecz M, Preininger K, Englisch R, *et al.* Acute effect of leptin on hepatic glycogenolysis and gluconeogenesis in perfused rat liver. *Hepatology* 1999; **29**: 166.
29. Borba-Murad GR, Mario EG, Bassoli BK, Bazotte RB, de Souza HM. Comparative acute effects of leptin and insulin

on gluconeogenesis and ketogenesis in perfused rat liver. *Cell Biochem Funct* 2005; **23**: 405.
30. Alpini G, Baiocchi L, Glaser S, *et al.* Ursodeoxycholate and tauroursodeoxycholate inhibit cholangiocyte growth and secretion of BDL rats through activation of PKC alpha. *Hepatology* 2002; **35**: 1041.
31. Huet PM, Nagaoka MR, Desbiens G, *et al.* Sinusoidal endothelial cell and hepatocyte death following cold ischemia-warm reperfusion of the rat liver. *Hepatology* 2004; **39**: 1110.
32. Bahde R, Spiegel HU. Hepatic ischaemia-reperfusion injury from bench to bedside. *Br J Surg* 2010; **97**: 1461.
33. Smith CC, Yellon DM. Adipocytokines, cardiovascular pathophysiology and myocardial protection. *Pharmacol Ther* 2011; **129**: 206.
34. Zhang JY, Yan GT, Liao J, *et al.* Leptin attenuates cerebral ischemia/reperfusion injury partially by CGRP expression. *Eur J Pharmacol* 2011; **3**: 61.
35. Deng Jr ZH, Yan GT, Wang LH, Zhang JY, Xue H, Zhang K. Leptin relieves intestinal ischemia/reperfusion injury by promoting ERK1/2 phosphorylation and the NO signaling pathway. *J Trauma* 2012; **72**: 143.
36. Fiorini RN, Shafizadeh SF, Polito C, *et al.* Anti-endotoxin monoclonal antibodies are protective against hepatic ischemia/reperfusion injury in steatotic mice. *Am J Transplant* 2004; **4**: 1567.
37. Hong F, Radaeva S, Pan HN, *et al.* Interleukin 6 alleviates hepatic steatosis and ischemia/reperfusion injury in mice with fatty liver disease. *Hepatology* 2004; **40**: 933.
38. Lin J, Gao XN, Yan GT, *et al.* Endogenous leptin fluctuates in hepatic ischemia/reperfusion injury and represents a potential therapeutic target. *World J Gastroenterol* 2010; **16**: 5424.
39. Terui K, Enosawa S, Haga S, *et al.* Stat3 confers resistance against hypoxia/reoxygenation-induced oxidative injury in hepatocytes through upregulation of Mn-SOD. *J Hepatol* 2004; **41**: 957.
40. Eguchi M, Liu Y, Shin J, Sweeney G. Leptin protects H9c2 rat cardiomyocytes from H2O2-induced apoptosis. *FEBS J* 2008; **275**: 3136.
41. Cusi K. Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis (Nash): pathophysiology and clinical implications. *Gastroenterology* 2012; **142**: 711.
42. Gujral JS, Bucci TJ, Farhood A, Jaeschke H. Mechanism of cell death during warm hepatic ischemia-reperfusion in rats: apoptosis or necrosis? *Hepatology* 2001; **33**: 397.
43. Jaeschke H, Lemasters JJ. Apoptosis versus oncotic necrosis in hepatic ischemia/reperfusion injury. *Gastroenterology* 2003; **125**: 1246.
44. Malhi H, Gores GJ, Lemasters JJ. Apoptosis and necrosis in the liver: a tale of two deaths? *Hepatology* 2006; **2**(Suppl. 1): S31.