Adipokines and Redox Signaling:
Impact on Fatty Liver Disease

Maurizio Parola[1] and Fabio Marra[2]

Abstract

Adipokines (adipose tissue cytokines) are polypeptide factors secreted by adipose tissue in a highly regulated manner. The 'classical' adipokines (leptin, adiponectin, and resistin) are expressed only by adipocytes, but other adipokines have been shown to be released by resident and infiltrating macrophages, as well as by components of the vascular stroma. Indeed, adipose tissue inflammation is known to be associated with a modification in the pattern of adipokine secretion. Several studies indicate that adipokines can interfere with hepatic injury associated with fatty infiltration, differentially modulating steatosis, inflammation, and fibrosis. Moreover, plasma levels of adipokines have been investigated in patients with nonalcoholic fatty liver disease in order to establish correlations with the underlying state of insulin resistance and with the type and severity of hepatic damage. In this Forum article, we provide a review of recent data that suggest a significant role for oxidative stress, reactive oxygen species, and redox signaling in mediating actions of adipokines that are relevant in the pathogenesis of nonalcoholic fatty liver disease, including hepatic insulin resistance, inflammation, and fibrosis. *Antioxid. Redox Signal.* 15, 461–483.

Adipokines

WHITE ADIPOSE TISSUE, the most abundant in adults, has three main functions: 1) storage of energy in the form of triglycerides; 2) hydrolysis of triglycerides to provide free fatty acids to support the energy needs of tissues, especially skeletal muscle; 3) release of adipokines (or adipocytokines). Data accumulated over the last two decades have shown that the functions of adipose tissue are not limited to energy storage, but extend to fundamental processes such as metabolism, immune response, tissue regeneration, wound healing, and cancer. In this context, the relations between the liver and adipose tissue are particularly close, and represent the result of a diversification of functions that are present in the same tissue in lower organisms (72). The interest in adipose tissue pathophysiology has grown along with the dramatic increase in the prevalence of obesity (116). Obesity is a component of the metabolic syndrome and thus a major risk factor for the development of nonalcoholic fatty liver disease (NAFLD) and for progression to advanced fibrosis and cirrhosis in the context of nonalcoholic steatohepatitis. Nonetheless, adipose tissue expansion has an impact on liver diseases in general, as cirrhosis is more prevalent in obese patients (139), and obesity favors the appearance of severe fibrosis in chronic liver diseases (3), and confers a higher risk of liver cancer (28).

Adipokines (adipose tissue cytokines) are polypeptide factors which are expressed significantly, although not exclusively, by adipose tissue in a regulated manner. In adipose tissue, adipokines modulate adipocyte differentiation and regulate lipid accumulation through autocrine mechanisms. This feature has a clear relevance in obesity that is associated with adipose tissue inflammation, and has been linked to the appearance of insulin resistance and to the metabolic and cardiovascular complications of the metabolic syndrome (153). An additional factor that has received considerable attention in the pathophysiology of adipose tissue is the role of ectopic fat, that is, adipose tissue expansion at sites different from subcutaneous adipose tissue, such as in the omentum (visceral fat) or around the heart (epicardial or mediastinal fat). Ectopic fat is more likely to undergo inflammation and to contribute to the pathogenesis of obesity-related disorders (72).

Only one-third of adipose tissue is composed of adipocytes, the rest being stromal cells, macrophages, fibroblasts, and monocytes, all of which contribute to adipokine production. The 'classical' adipokines are those primarily expressed by adipocytes, namely leptin, adiponectin, and resistin, but an expanding group of newly identified adipokines is currently

[1]Dipartimento di Medicina e Oncologia Sperimentale and Centro Interuniversitario di Fisiopatologia Epatica Università degli Studi di Torino, Turin, Italy.
[2]Dipartimento di Medicina—Center of Research, Transfer and Higher Education DenoTHE, Università degli Studi di Firenze, Florence, Italy.

being investigated and some of them may be expressed by vasculo-stromal cells (117).

Leptin has been the first adipokine to be identified, as the circulating product of the *obese (ob)* gene, which is expressed by adipose tissue and at several other sites (129). Leptin receptors belong to the class I of cytokine receptor superfamily, and six isoforms have been identified. The long receptor, ObRb, mediates most of the biological effects of this adipokine, and is able to fully activate intracellular signaling via the Jak-2/Stat3 pathway (129). Secretion of leptin is directly proportional to the fat mass, and provides anti-obesity signals, regulating food intake and energy expenditure in conditions of energy excess, through hypothalamic pathways. This action is reflected by the phenotype of *ob/ob* mice, which lack functional leptin and are obese and hyperphagic. In addition, leptin stimulates wound repair, modulates innate and adaptive immunity, and regulates hematopoiesis and reproduction.

Adiponectin circulates as a full-length molecule assembled in complexes of different molecular weight, or as the isolated C-terminal globular domain (85). Adiponectin binds at least two specific receptors, AdipoR1 and AdipoR2, (38) which belong to the seven transmembrane domain receptor family but are not coupled to G protein. AdipoR1 is expressed in several tissues and in skeletal muscle, while AdipoR2 is mostly expressed by hepatocytes. Binding experiments indicate that AdipoR1 interacts preferentially with the globular domain, and its main downstream effector is the AMP-activated protein kinase (AMPK). AdipoR2 binds with similar affinity both full-length and globular adiponectin and signals predominantly via activation of peroxisome proliferator-activated receptor-α (PPARα).

Adiponectin concentrations are inversely correlated with fat mass, and are downregulated in patients with obesity (85). Moreover, adiponectin exerts insulin sensitizing effects and acts as an anti-inflammatory molecule. Conversely, inflammation is a potent inhibitor of adiponectin release, and adipose tissue inflammation is considered one of the main mechanisms underlying reduced plasma levels in obesity.

Resistin belongs to a family of small cysteine-rich secretory proteins, named FIZZ (found in inflammatory zone) or RELMs (resistin-like molecules) (166). In rodents, resistin is highly expressed by adipose tissue and circulating levels are increased during diet-induced or genetic obesity (165), where resistin may be a link between obesity and insulin resistance (137). Reduced hepatic glucose production in the absence of resistin is also associated with higher hepatic AMPK activation (14). In humans, the biology of resistin is less defined, and most studies demonstrate that resistin is expressed predominantly by bone marrow-derived cells and inflammatory cells (128). In human monocytes, resistin expression is increased by treatment with proinflammatory cytokines *in vitro* and circulating levels of resistin are higher in different conditions of inflammation.

Adipokines, Oxidative Stress, and Hepatic Insulin Resistance

Insulin signaling pathways and the hepatic control of glucose and lipid metabolism

Hepatocytes, together with myocytes and adipocytes, represent a major cellular target for insulin by expressing a high number of insulin receptor (IR) molecules on their plasma membrane (32, 175). The most relevant aspects of the insulin signaling network will be briefly summarized herein and in Figures 1 and 2, dedicated to the overall control of insulin signaling (110, 123, 175, 187) and related cellular responses (175), respectively. Insulin, binding to one of the two isoforms of its tetrameric receptor (IR$_A$ or IR$_B$, having different affinity for insulin and IGF1), activates signaling by leading to de-repression of tyrosine-kinase (TK) activity of the two intracellular β-subunits and to trans-phosphorylation of specific tyrosine residues, resulting in a further increase in kinase activity.

Following activation of IR (and IGFR1), the insulin signaling network, which is carefully controlled by a number of mechanisms (illustrated in Fig. 1, Ref.175), proceeds through phosphorylation of intracellular substrate proteins, including the six different isoforms of insulin receptor substrate (IRS) proteins (IRS 1–6), and various isoforms of Src homology-2 (SH2)-containing proteins (175), that in turn, mediate the binding of downstream effectors. Best characterized and most relevant for insulin signaling are IRS1 and IRS2 (widely distributed, major isoforms in hepatocytes), IRS3 (mostly restricted to adipose tissue and brain), and IRS4 (expressed in embryos and cell lines). Phosphorylated IRS serve as docking platforms for signaling proteins containing SH2, including: 1) the regulatory subunit of phosphatidylinositol 3-kinase (PI3K) or the adaptor Grb2, which activate the Ras/MAPK signaling pathway; 2) enzymes like SH2-domain containing tyrosine phosphatase-2 (SHP2), cytoplasmic tyrosine kinases, and the Ca2+-ATPases SERCA1 and 2.

IR activation, through the involvement of IRS, results in the engagement of two main divergent pathways (Fig. 2; 175): 1) PI3K, leading to activation of PKB/Akt or atypical PKC isoforms, a pathway which mediates the main metabolic actions of insulin with a complexity that is exacerbated by the existence of multiple isoforms of IRS, PI3K, (regulatory and catalytic) and Akt (reviewed in Ref. 175); 2) the MAP kinase pathway, leading to cell proliferation, survival, and differentiation.

In hepatocytes (67, 99, 175), IRS-1 has a major role in handling increased glucose levels in the fed state and is involved in the control of glycogen synthesis and lipogenesis. Glucose, entering hepatocytes through the specific GLUT-2 transporter, is then directed to the glycolytic pathway and ATP production in order to fulfill energy requirements and replenish glycogen stores. Excess glucose can be diverted to lipogenesis, and insulin can then act as a potent stimulator of lipogenic pathways by operating mainly through sterol regulatory element binding protein-1 (SREBP-1) and the glucose-responsive transcription factor carbohydrate response element-binding protein (ChREBP) (135, 178). IRS2 is more involved in the fasting state, when it is upregulated to favor the action of insulin, limiting the expression of gluconeogenic enzymes such as glucose-6-phosphatase or phosphoenolpyruvate carboxykinase (PEPCK) and then the hepatic production of glucose and its export by the GLUT2 transporter. Besides the opposing effects of insulin and glucagon on fatty acid mitochondrial oxidation, insulin signaling limits glucagon-stimulated gluconeogenesis by means of Akt–dependent phosphorylation of the forkhead box–containing protein O subfamily-1 (FOXO1) transcription factor. When phosphorylated, FOXO1 is segregated into the cytoplasm and degraded by the proteasome pathway, preventing nuclear translocation and regulation of PEPCK expression (99, 135).

FIG. 1. Insulin signaling pathway and its regulatory mechanisms. The figure shows the major steps in insulin-mediated signaling following interaction of insulin with the tetrameric insulin receptor (IR) complex at the plasma membrane level, together with the major inhibitory mechanisms and the cross-talk with signaling pathways elicited by cytokines and growth factors, including TNFα and IGF-1. Following ligand–receptor interaction, IR activation is tightly regulated by several negative mechanisms that include (175): a) tyrosine phosphatases, particularly PTP1B that directly interacts with IR to dephosphorylate critical tyrosine residues; b) proteins that sterically block IR function either by preventing its interaction with intracellular substrate proteins or by modifying its kinase activity, including

suppressor of cytokine signaling (SOCS)-1 and −3, which are known to be upregulated under conditions of insulin resistance; and c) ligand-stimulated internalization and degradation of IR, a process which again occurs under conditions of insulin resistance.
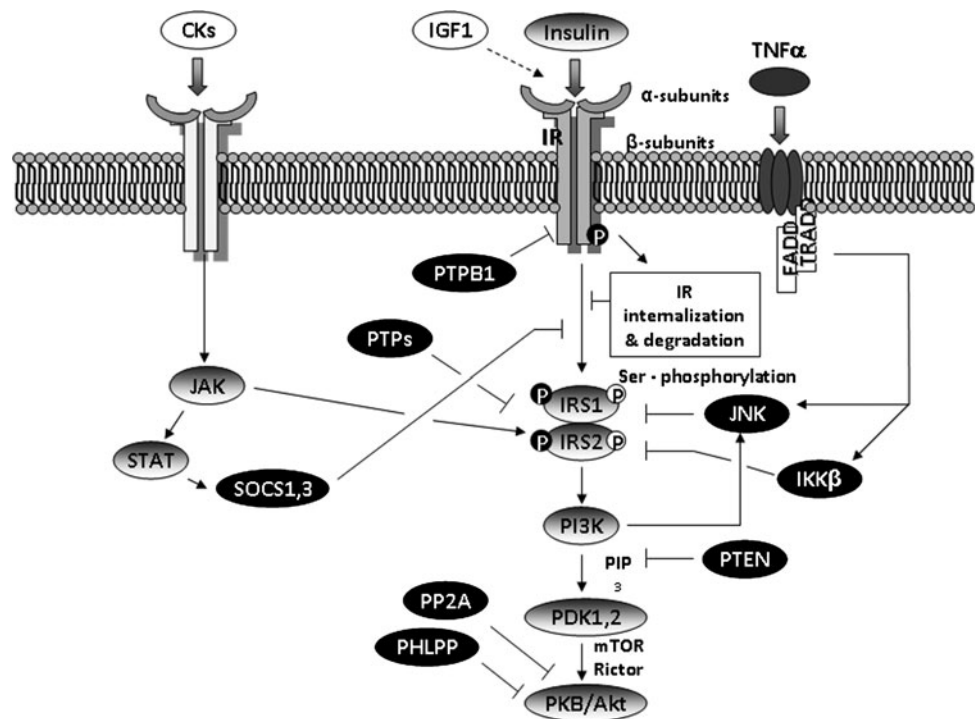
Upon activation, IR as well as IGF1R can phosphorylate intracellular substrate proteins that mediate the binding of downstream effectors, the most relevant being the isoforms of insulin receptor substrate (IRS) proteins, leading then to the activation of PI3K and PKB/Akt. PI3K acts as a negative regulator of insulin signaling on the basis of at least three distinct mechanisms: 1) efficiency of insulin signaling critically depends on the stoichiometry of the p85 regulatory subunit to the catalytic heterodimer (123); 2) sequestration of IRS/PI3K complex into signaling-silent compartments (*i.e.*, incapable of generating PIP3) provides negative regulation (110); 3) the recruitment of inhibitory proteins that interact with monomeric p85α regulatory subunits. This leads to either PIP3 degradation by phospholipid phosphatases like PTEN or through negative regulation of insulin signaling by activation of JNK activity. In particular, JNK activation, which is also induced by TNF, operates its inhibitory action phosphorylating IRS1 and IRS2 on serine residues (175, 187). A very close mechanism has been described for IκB kinase beta (IKKβ) that controls the activation of NF-κB; once activated, IKKβ phosphorylates IRS-1 on Ser307 residue, resulting in inhibition of insulin action (63). Further mechanisms leading to inhibition of insulin signaling are operated through suppressors of cytokine signaling (SOCSs), regulated by several cytokines, including IL-6 and leptin, and upregulated in conditions of insulin resistance (135, 175, 178). SOCS proteins act by linking IRS to ubiquitin-mediated degradation pathways and activating SREBP1, thus favoring lipogenesis (154, 188). Similar SOCS-mediated mechanisms may operate in insulin resistance associated with chronic HCV infection, as HCV core protein can upregulate SOCS and then promote degradation of IRS1 and IRS2 (91).

Akt is negatively regulated by the action of the phosphatases Src-homology-2 domain-containing inositol phospatase-2A (PP2A) and the pleckstrin-homology (PH) domain leucine-rich-repeat protein phosphatase (PHLPP). Other details can be found in the text.

As far as PI3K and Akt are concerned, IR- or IGF1R-related PI3K involvement results from direct interaction with phosphorylated substrate proteins, including IRS, leading to phosphatydylinositol-3-phosphate (PIP$_3$) generation and, in turn, PIP$_3$-mediated activation of different Akt isoforms. Apart from negative feedback control on insulin signaling by PI3K (see Fig. 1), PIP3 recruits phosphoinositide-dependent kinase-1 and Akt isoforms at the plasma membrane, where the different Akt isoforms (Akt-1, −2, and −3) are phosphorylated on tyrosine and serine residues by PDK1 and PDK2, possibly through formation of a complex with the mammalian target of rapamycin (mTOR). Phosphorylation of Akt in turn confers the ability to phosphorylate a series of downstream signaling targets that mediate major effects of insulin signaling on glycogen synthesis, gluconeogenesis, and protein synthesis (175).

Hepatic insulin resistance and its effects on glucose and lipid metabolism

It has been proposed that hepatic insulin resistance represents the single major pathophysiologic derangement in the metabolic syndrome (95) as well as a major culprit in the development of type II diabetes (59, 184). Along these lines, non-alcoholic fatty liver disease (NAFLD) is strongly associated with both hepatic and adipose tissue insulin resistance, and reduced whole body insulin sensitivity (26, 115, 156). Moreover, NAFLD patients also exhibit a reduction in fatty acid oxidation, a feature which is likely to reflect a decreased uptake and utilization of glucose as a source of energy (26). All these findings have led to the suggestion (see Ref. 135) that insulin resistance may represent an intrinsic defect in NAFLD and that the decreased insulin responsiveness at the level of adipocytes may significantly
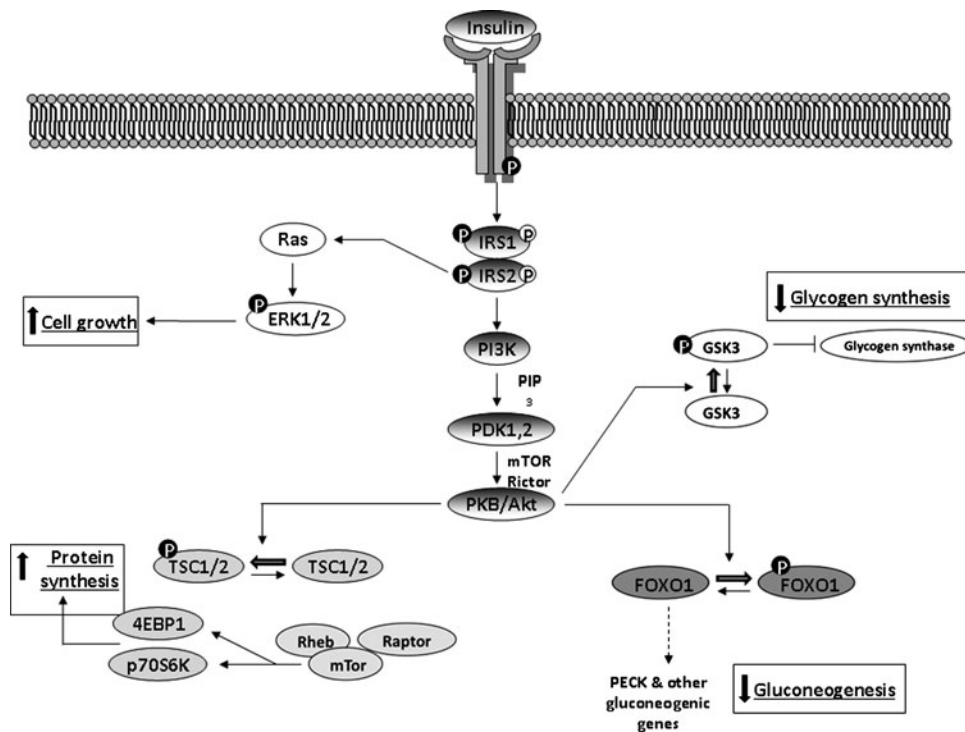
FIG. 2. Insulin signaling and related metabolic or functional responses. IR activation, through the involvement of IRS, results in the engagement of two main and divergent pathways (175). First, the MAP kinase pathway, particularly Ras/Erk, leads to cell proliferation, survival, and differentiation. Second, the PI3K pathway leads to activation of PKB/Akt or atypical PKC isoforms, which mediates the main metabolic actions of insulin. Activation/phosphorylation of PKB/Akt, in particular, confers the ability to phosphorylate a series of downstream signaling targets that mediate major effect of insulin signaling on glycogen synthesis, gluconeogenesis, and protein synthesis: a) glycogen-synthase kinase 3 (GSK3), that when phosphorylated is inactive on glycogen synthase, leading to increased glycogen synthesis; b) FOXO1, a transcription factor which is phosphorylated on Ser256 and sequestered in the cytoplasm, thus preventing its action on genes related to gluconeogenesis like PEPCK; c) Akt substrate of 160 kDa (AS160), which in turn controls the activity of Rab-GTpase activating protein and translocation of glucose transporters (*e.g.*, GLUT4) to plasma membrane; d) tuberin or tuberous sclerosis complex-2 (TSC2) which is complexed with TSC-1: phosphorylation of TSC1/2 by PKB/Akt removes the TSC1/2-mediated inhibitory control on the mTOR pathway, leading to phosphorylation of mTOR downstream signaling substrates like the eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) and p70 ribosomal protein S6 kinase (p70S6K), leading to upregulation of protein synthesis.

contribute to hepatic steatosis, providing an excess of FFA flux to the liver. According to the scope of this Forum article, we will focus on the pathophysiology of impaired glucose and lipid metabolism at hepatic level, considering the liver as a major site of insulin action, concentrating our attention on those adipokine- and/or oxidative stress-related mechanisms or molecular derangements associated with hepatic insulin resistance that are also likely to favor the development of liver steatosis.

The major and well-established general problem with insulin resistance is a significant decrease in the ability of insulin to stimulate glucose disposal and inhibit hepatic glucose production (86). It has been suggested (135) that humans can accumulate the excess of FFA as TG stores as a consequence of a limited number of events, including: a) reduction of fatty acid oxidation; b) increased *de novo* lipogenesis; c) increased fatty acid hepatic influx; d) impaired fatty acid and TG efflux from the liver. Moreover, peripheral (*i.e.*, skeletal muscle and adipose tissue) insulin resistance results in an increased flux of FFA towards the liver and then favoring hepatic steatosis (135). Finally, as we will detail later, one should remember that hepatic steatosis itself can further result in insulin resistance and exacerbate whole body insulin resistance (92, 93).

Adipokines, oxidative stress and inflammation: molecular links to hepatic insulin resistance

A derangement or loss of insulin signaling at the hepatocyte level has been proposed to lead to severe insulin resis-

tance and progressive hepatic dysfunction, and is considered a major mechanism for impaired glucose handling and increased fat storage, not only for the liver but also for skeletal muscle and adipose tissue (124). Here we will focus on the role of the adipokines and oxidative stress, and particularly on the role of intracellular levels of reactive oxygen species (ROS), both as mediators and modulators of critical signaling event. An extensive introduction on ROS, antioxidant defenses, and principles of redox signaling is of course out of the scope of the present Forum article and the interested reader can refer to more comprehensive and specialized reviews (37, 132, 177) as well as to Figures 3–6 and related legends which offer, in this connection, a synthetic overview of most relevant concepts. In this review we will try instead to emphasize established and putative redox mechanisms that may be based on ROS or other oxidative stress-related reactive intermediates generated within the cell or entering target cells (mainly hydrogen peroxide and 4-hydroxy-2,3,-nonenal or HNE), that are generated in a pro-oxidant microenvironment.

Intracellular oxidative stress can occur in fat-laden hepatocytes. As reviewed elsewhere (133, 135) increased hepatocyte levels of FFAs in NAFLD/NASH are considered a relevant mechanism leading to hepatocyte injury: a) FFAs can directly induce hepatocyte apoptosis and stimulate production of TNF, which in this context should also be considered as an adipocytokine; b) FFAs can increase Fas ligand binding to the Fas receptor (CD95) in steatotic hepatocytes, leading to apoptosis; c) excess of FFAs in hepatocytes leads to impaired mitochon-
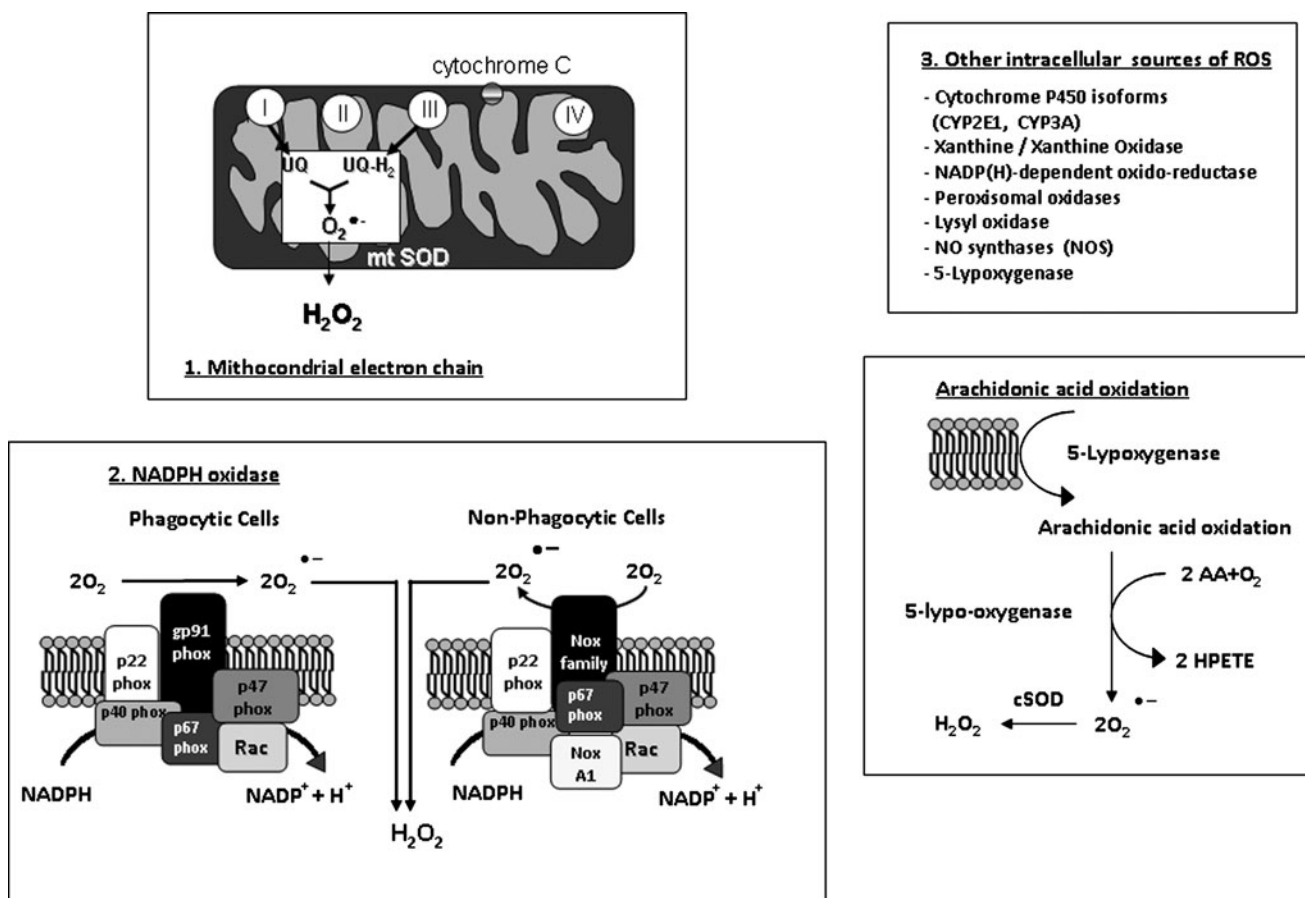
FIG. 3. Intracellular generation of ROS in mammalian cells. ROS can be generated within living cells by the following major sources: 1) Mitochondria: Approximately 1%–5 % of the electrons flowing through the electron transport chain can be diverted to form $O_2^{\bullet-}$ at the level of Complex I (NADH/ubiquinone oxidoreductase) and Complex III (ubiquinol/cytochrome-c oxidoreductase). $O_2^{\bullet-}$ is then converted by a mitochondrial isoform of superoxide dismutase (mtSOD) into H_2O_2 that can cross mitochondrial membranes and then reach the cytoplasm. 2) Plasma membrane NADPH oxidase (NOX): This multi-subunit complex is expressed by professional phagocytic cells (macrophages, neutrophils, and eosinophils) as well as by a number of nonphagocytic cells playing a critical role in human diseases. NOX of professional phagocytes and of nonphagocytic cells are similar in their structure being formed by two membrane bound components (p22phox and gp91phox/Nox2 or another member of the NOX family of protein) forming the flavocytochrome b558, and four cytosolic components (p40 phox, p47phox, p67phox, and the GTPase Rac1/2), that following stimulation, are recruited to the plasma membrane where they interact with Cyt b558 leading to increased activity and then generation of $O_2^{\bullet-}$ that is then converted into H_2O_2. Where redox signaling is concerned, the major difference is that nonphagocytic NOX, which is constitutively active and produce a very low level of ROS, can significantly increase both activity and ROS generation in response to growth factors, cyto- and chemokines, and other conditions. 3) Several enzymes involved in redox reactions: Several enzymes are able to generate ROS (mostly $O_2^{\bullet-}$ that is rapidly converted by a SOD isoform into H_2O_2) during their catalytic activity, including several oxidases, peroxidases, cytochromes, mono- and di-oxygenases, with the following being the most relevant examples: isoforms of the cytochrome P450 superfamily, involved in metabolism of endo- and xenobiotics, including ethanol, steroid hormones, drugs, and chemotherapeutics; xanthine oxidase; the isoforms of cyclooxygenase; peroxisomal oxidases, that can generate directly H_2O_2 when metabolizing various substrates (glycolate-, D-amino-, ureate-, fatty acid-CoA-, and L-α-hydroxyacid oxidases); lysyl oxidase that again generate H_2O_2 when catalyzing the formation of aldehyde precursors of cross-links in collagen and elastin; 5-lipoxygenase, a mixed function oxidase involved in the synthesis of leukotrienes from arachidonic acid in response to stimuli that are also able to stimulate NOX, particularly growth factors and cytokines.

drial or peroxisomal oxidation of FFAs, eventually leading to generation of ROS and products of lipid peroxidation, mainly HNE, which in turn may cause cell injury and death; d) induction of ER stress and of the 'unfolded protein response', which potentially results in the induction of caspase-dependent cell death involving mitochondria (71, 133, 135, 136, 178). It should be noted that all these mechanisms are intrinsically related to increased intracellular ROS generation (Fig. 3).

A first redox-dependent mechanism involved in insulin resistance is based on the activation of c-jun N-terminal kinase (JNK). Both TNF and FFAs are powerful modulators of the activity of two kinases, namely JNK and IκB kinase (IKK), which couple inflammatory and metabolic signals (133, 178). Interaction of TNF with its receptor results in activation of NADPH oxidase, mitochondrial outer membrane depolarization, and FFA-related mitochondrial dysfunction, ER stress
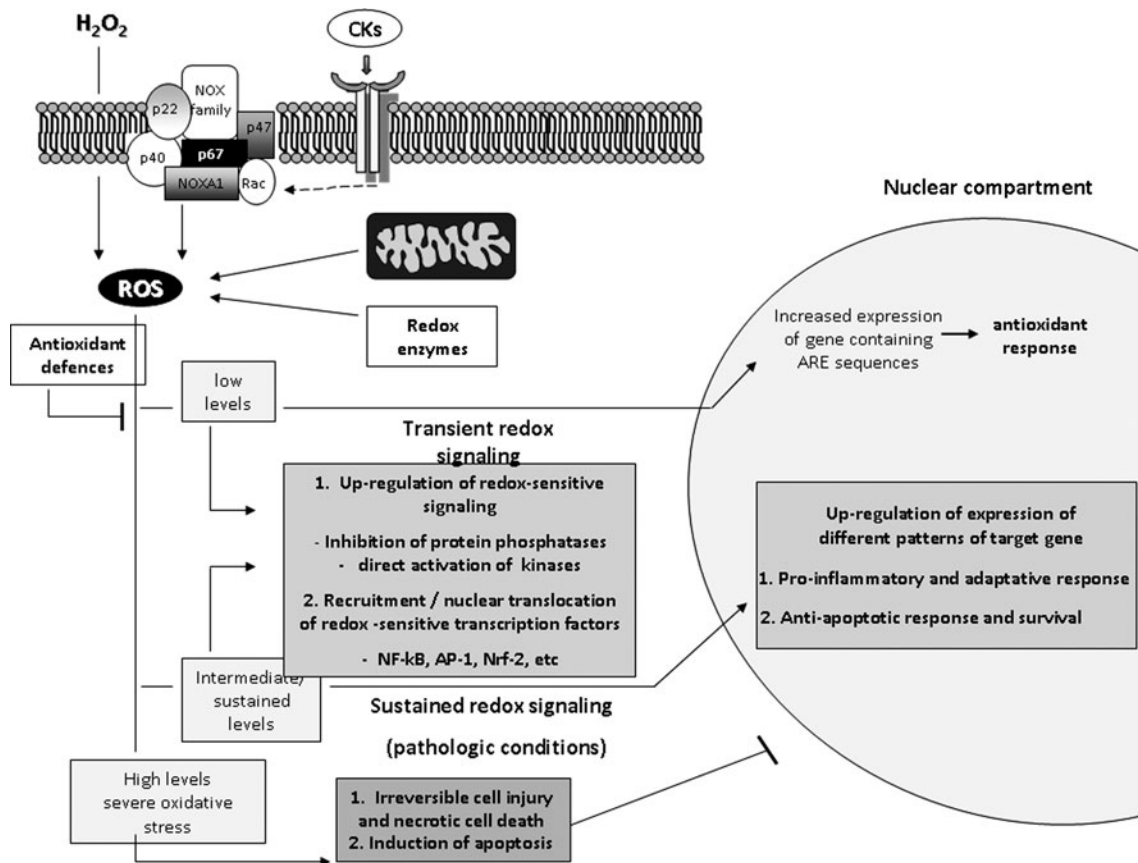
FIG. 4. Simplified scheme of redox signaling induced by increase in intracellular levels of ROS. Under physiological conditions, cells and tissues of aerobic organisms have to face relatively low amount (steady-state levels) of ROS, free radicals, and other reactive intermediates that are the result of a dynamic balance between the rate of their generation and the rate of their removal by one or more of defensive antioxidant systems (see Fig. 5), including highly specialized enzymes (catalase, thioredoxins, SODs, and GPXs), naturally occurring antioxidants (GSH, vitamin E, β-carotene, ascorbate, urate, and many others) as well as by amino acids, peptides, and proteins. Under these conditions, there is no significant unbalance of pro-oxidants vs. antioxidant defenses and thus no response by means of a redox signaling.

Whenever redox homeostasis is significantly disturbed, by an increase in ROS generation (whatever the source, intra- or extracellular), by a decrease in one or more antioxidants, or by a change in the thiol/disulfide redox state, redox signaling can be elicited. This can potentially lead to at least three different scenarios in which the difference is made primarily by the absolute levels of ROS and other reactive species reached within the cell as well as by the temporal length of the alteration. If the starting stimulus/condition is able to induce a relatively low and transient (*i.e.*, time limited) increase in intracellular levels of ROS and other reactive mediators, then also the shift in redox balance will be limited and redox signaling will operate through defined redox-sensitive signaling pathways and transcription factors to upregulate mainly transcription of genes able to encode for products that will reset in the due time redox homeostasis (for example, antioxidant enzymes, Trxs and Glrxs, cystine transport system to sustain genesis of GSH).

However, in conditions of extensive acute tissue injury as well as in tissues undergoing persisting injury, chronic inflammation, and chronic wound healing, levels of ROS and other related reactive intermediates or reactions (produced within the "target cells" or by extracellular sources, such as by inflammatory or damaged cells) may be very high and/or persistently increased within the cell to overcome antioxidant defenses and/or antioxidant response. If levels of ROS and related reactive intermediates are very high, this will lead to irreversible injury and necrotic—or apoptotic—type of cell death. Alternatively, when levels of oxidative stress are significantly high but not overtly able to induce irreversible cell damage, as in chronic inflammatory diseases, cells and/or tissues may still reach an altered equilibrium characterized by a shift of the intracellular redox state to higher levels of ROS and a chronically dysregulated state in which redox signalling is upregulating different patterns of gene expression and cell responses, then contributing to the progression of the disease.

and/or increased activity of cytochrome P450 isoforms like CYP2E1 and CYP3A (Fig. 2). On the other hand, ROS can activate JNK (177, 196) either through activation of the upstream apoptosis-stimulated kinase 1 (ASK) or inactivation of specific JNK-phosphatases (177, 196). Similarly, HNE has been shown to interact directly with JNK, leading to its activation (133). In the context of hepatic insulin resistance, activation of JNK, and particularly of JNK-1, leads to increased phosphorylation of IRS1 on serine 307, preventing its binding to the IR and propagation of insulin signaling (4, 73, 185). Upregulation of this inhibitory mechanism in association with insulin resistance should be considered as an exacerbation of a negative feed-back mechanism for the insulin signaling pathway (175), since insulin itself is able to activate JNK and
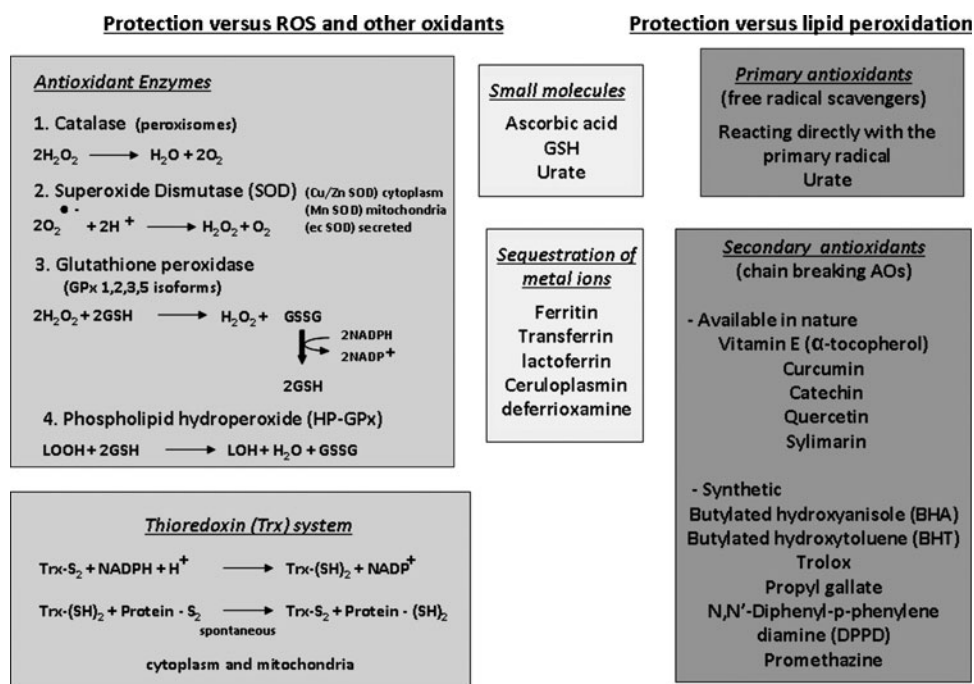
Protection versus ROS and other oxidants

Protection versus lipid peroxidation

Antioxidant Enzymes

1. Catalase (peroxisomes)

$$2H_2O_2 \longrightarrow H_2O + 2O_2$$

2. Superoxide Dismutase (SOD) (Cu/Zn SOD) cytoplasm
   (Mn SOD) mitochondria
   (ec SOD) secreted

$$2O_2^{\bullet-} + 2H^+ \longrightarrow H_2O_2 + O_2$$

3. Glutathione peroxidase
   (GPx 1,2,3,5 isoforms)

$$2H_2O_2 + 2GSH \longrightarrow H_2O_2 + GSSG$$

2NADPH
2NADP$^+$

2GSH

4. Phospholipid hydroperoxide (HP-GPx)

$$LOOH + 2GSH \longrightarrow LOH + H_2O + GSSG$$

Small molecules

Ascorbic acid
GSH
Urate

Sequestration of metal ions

Ferritin
Transferrin
lactoferrin
Ceruloplasmin
deferrioxamine

Primary antioxidants
(free radical scavengers)

Reacting directly with the primary radical
Urate

Secondary antioxidants
(chain breaking AOs)

- Available in nature
  Vitamin E (α-tocopherol)
  Curcumin
  Catechin
  Quercetin
  Sylimarin

- Synthetic
  Butylated hydroxyanisole (BHA)
  Butylated hydroxytoluene (BHT)
  Trolox
  Propyl gallate
  N,N'-Diphenyl-p-phenylene diamine (DPPD)
  Promethazine

Thioredoxin (Trx) system

$$Trx \cdot S_2 + NADPH + H^+ \longrightarrow Trx \cdot (SH)_2 + NADP^+$$

$$Trx \cdot (SH)_2 + Protein \cdot S_2 \longrightarrow Trx \cdot S_2 + Protein \cdot (SH)_2$$

spontaneous

cytoplasm and mitochondria

FIG. 5. Synopsis of major antioxidant defenses in a mammalian cell. Antioxidant defenses relies on the sum of those mechanisms that nature has developed to protect biological materials from ROS and other oxidants and those designed to protect them from lipid peroxidation. In order to simplify concepts, antioxidant defenses may be differentiated into two major (although inter-related) categories, separating defenses affording protection versus ROS and other oxidants from those mainly offering protection against lipid peroxidation. Within the first category we can include the following class of defenses:

1. *Antioxidant enzymes.* This definition includes a group of very specific enzymes such as catalase, glutathione peroxidase (GPX) isoforms, and superoxide dismutase (SOD) isoforms. Catalase and GPX isoforms are responsible for the removal of hydrogen peroxide but also of other organic hydroperoxides, whereas SOD isoforms operate by transforming superoxide into hydrogen peroxide. Major reactions of these enzymes are described.
2. *Small molecules.* This definition applies to several molecules but by far the most relevant are: a) ascorbic acid (vitamin C), a molecule that can act as an electron donor and then as a reducing agent; ascorbate can also scavenge (*i.e.*, interact directly with) •OH; b) *reduced glutathione* (GSH) that is a hydrosoluble tripeptide able to act as substrate for an enzyme able to remove H_2O_2 like GPX, as a scavenger of •OH and singlet oxygen, or as a low molecular weight thiol in regenerating oxidized –SH groups of proteins; in the figure, the essential reaction of GSSG reductase, designed to recover GSH, is also shown; c) *uric acid*, present in blood plasma, that has been reported to scavenge singlet oxygen, •OH, and peroxy radicals.
3. *Protection by sequestration of metal ions.* Transition metal ions such as iron and copper are able to lead to generation of very reactive species from less reactive ones. Then a number of metalloproteins such as ferritin, transferrin, metallothionein, and lactoferrin can be seen not only as relevant for their respective role in metal homeostasis but also as molecules that by "sequestering" redox active metal ions may prevent ROS production via the Fenton reaction.
4. *Thioredoxin and glutaredoxin systems.* Thioredoxins (Trxs), described in the figure, are small proteins that have a catalytic site containing two cysteine residues which can be oxidized reversibly to form disulfide bridges. They can undergo NADPH-dependent reduction by the enzyme thioredoxin reductase, and in turn they can reduce oxidized cysteine groups on proteins. These proteins, according to this intramolecular disulfide–thiol exchange, can act as hydrogen donors contributing to the control of redox state and redox signaling (for example, by affecting the regulation of kinases or transcription factors forming with them heterodimers).

Glutaredoxins (Glrxs) also belong to the thioredoxin superfamily of thiol/disulfide exchange proteins and in biological systems serve as reductants of protein–SG mixed disulfides and. similarly to what is described for thioredoxin system the glutaredoxin system (composed of Glrx isoforms, by glutathione reductase, GSH, and again NADPH) are also involved in redox signaling.

Where protection from lipid peroxidation is concerned, two classes of antioxidants can be described:

1. *Primary antioxidants,* also often defined as "free radical scavengers" because they are able to interact directly with, and/or to block the initiating free-radical (as for example •OH); examples are urate (able to scavenge peroxy- and alkoxy radicals as well as HOCl), and glucose (able to scavenge •OH with a rate constant similar to that of mannitol).
2. *Secondary or chain breaking antioxidants,* (α-tocopherol or vitamin E being the prototype) which are able to intercept radical intermediates produced during on-going lipid peroxidation such as peroxyl- or alkoxyl-radicals, then preventing (*i.e.*, "breaking") the perpetuation of hydrogen abstraction in the chain reaction.
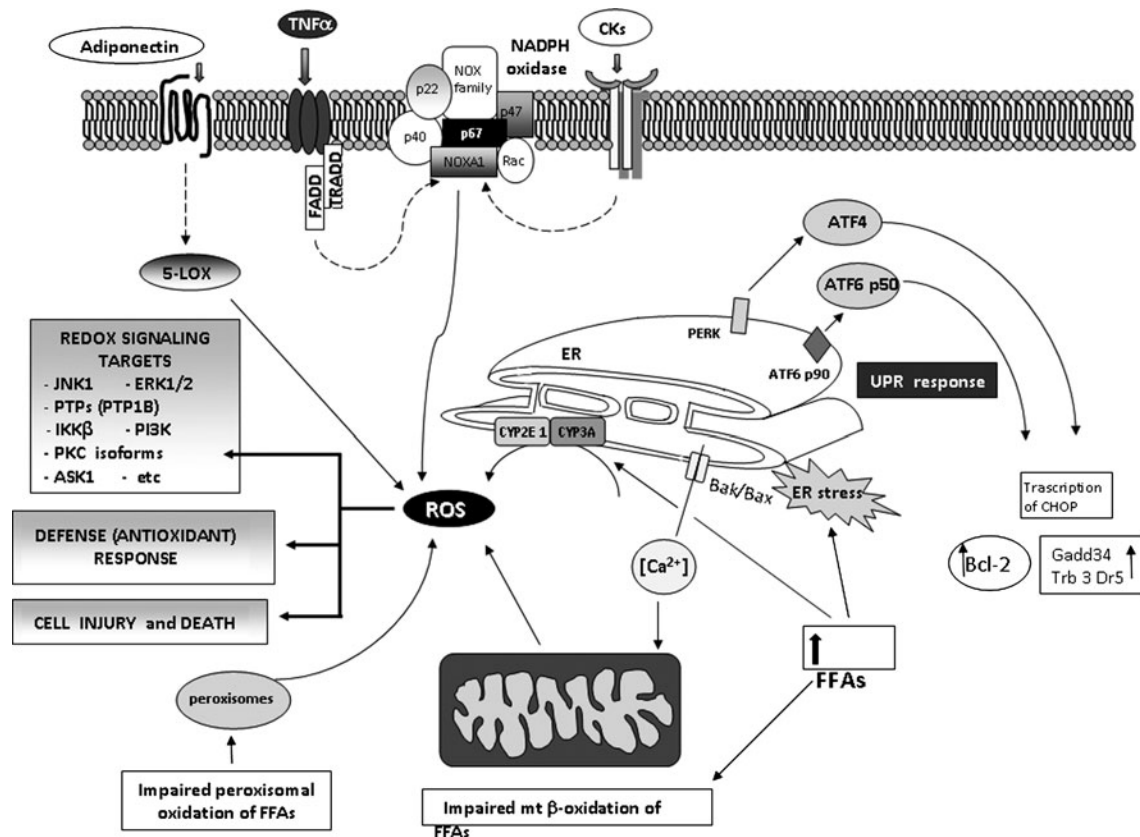
FIG. 6.   Generation of intracellular reactive oxygen species and related consequences in fat-laden hepatocytes. In the peculiar hepatic milieu of a NAFLD/NASH patient, reactive oxygen species (ROS) can be generated within fat-laden hepatocytes by a number of mechanisms that, pertinent to this review, may include the following: 1) Activation of NADPH oxidase that is known to parallel interaction of several cytokines to their cognate receptors, including also TNFα; 2) activation of 5-LOX by adiponectin following interactions with its receptor; 3) impairment of mitochondrial β-oxidation of free fatty acids (FFAs); 4) impairment of peroxisomal oxidation of FFAs; 5) induction of endoplasmic reticulum (ER) stress by increased intracellular levels of FFAs and/or related upregulation of CYP2E1 and CYP3A isoforms of the cytochrome P450 family. The scheme, that also indicates major steps of FFAs induced, ER stress-related UPR response, recapitulates major consequences of increased intracellular levels of ROS. Depending on the actual levels of intracellular ROS, the cell response may be limited to increased redox signaling (as exerted by redox-dependent modulation of several signaling components) and upregulation of antioxidant defenses (*i.e.*, the so-called antioxidant response). Excess generation of intracellular ROS may lead to cell injury and death. More details can be found in the text.

other kinases which phosphorylate IRS on serine residues (see Fig. 1). However, to emphasize the multiple role of redox signaling, one should remember that in parallel, insulin has been shown also to activate NADPH oxidase, leading to a transient increase in ROS generation that through inhibition of PTP1B transiently enhances insulin signaling (112).

The redox sensitivity of JNK1 is likely to be critical: JNK activation not only prevents interaction of the IR with serine-phosphorylated IRS proteins but also results in caspase activation and induction of apoptosis (41). Moreover, JNK1 is likely also to control AP-1-dependent transcription, increasing the expression of proinflammatory cytokines and further contributing to JNK activation. JNK may have also a role in activation of the SREBP1 pathway, known to be involved in lipogenesis, which is increased in the presence of insulin resistance, while it should theoretically be decreased (64, 147). Several explanations have been proposed for this apparent paradox, including: a) the pathway leading to SREBP-1 activation may remain sensitive to insulin; b) the activation of

lipogenesis by SREBP-1 and ChREB is sensitive to insulin as well as to high glucose levels; c) SREBP-1 can be activated by an insulin-independent mechanism related to ER-stress, that develops in liver steatosis (57, 83); d) SREBP-1 can be also activated by TNF as an additional contributory mechanism to increased hepatic lipogenesis (103).

Finally, JNK1 may have an additional role in regulating the expression and/or nuclear translocation of FOXO1, which is involved in gluconeogenesis. In this connection, insulin resistance results in a decrease of glycogen storage in hepatocytes in the postprandial state and in increased hepatic production of glucose (gluconeogenesis) in the fasting state (147). Data in experimental models indicate that fatty liver may directly induce hepatic insulin resistance by stimulating gluconeogenesis and activating PKC-ε and JNK-1, which may in turn impair the ability of insulin to activate glycogen synthase (144). Inhibition of hepatic glucose output by insulin involves Akt-dependent phosphorylation of FOXO1, a transcription factor controlling the expression of G6PC and

PEPCK (122, 130). Although NAFLD is characterized by hyperinsulinemia, under conditions of oxidative stress, as observed in NASH (43), FOXO1 becomes unresponsive to insulin because of interaction with the deacetylase sirtuin-1, resulting in induction of gluconeogenic genes (61, 131). The role of FOXO1 is supported by a recent study performed in control subjects and in patients with NAFLD or NASH (189), where it was found that: 1) expression of PEPCK is higher in patients with NASH than in those with simple NAFLD or normal liver, and was correlated with insulin resistance; 2) FOXO1 mRNA levels are higher in NASH and correlate with PEPCK; 3) in the presence of oxidative stress FOXO1 upregulation in steatohepatitis is associated with decreased Ser256 phosphorylation, with decreased Akt1 and increased JNK-1 activity.

Several kinases other than JNK-1 have been reported to be involved in insulin resistance as a possible consequence of increased generation of ROS and/or reactive nitrogen species (RNS) in response to a variety of stimuli, including hyperglycemia, elevated FFAs, or cytokines (52, 79). A critical example is represented by IκB kinase beta (IKKβ), a well known stress-sensitive kinase that controls the activation of NF-κB. Similar to other kinases, activated IKKβ phosphorylates IRS-1 on Ser307 residue, resulting in inhibition of insulin action (63). Interestingly, inhibitors of IKKβ such as salicylate, or ligands of peroxisome proliferator-activated receptor-γ (PPAR-γ) can restore insulin sensitivity (94, 208). Moreover, IKKβ (+/-) mice are more insulin-sensitive when compared to their control littermates (94, 208). In addition, treatment of a limited number of type 2 diabetes patients with high dose of aspirin resulted in reduced hepatic glucose production and fasting hyperglycemia as well as in increased insulin sensitivity (52).

Other kinases reported to be involved in oxidative stress-induced insulin resistance include p38 mitogen-activated protein kinase (p38MAPK), whose activation by oxidative stress inhibits insulin-stimulated glucose transport, as well as activation of mTOR, of several PKC isoforms (mainly PKCθ and PKCδ) and salt-inducible kinase 2 (SIK2) (reviewed in Ref. 52).

The action of adiponectin, a 'classical' adipokine, offers another example of a link between oxidative stress and insulin signaling. In the liver, adiponectin leads to AMPK phosphorylation that in turn causes decreased expression of genes involved in gluconeogenesis (e.g., glucose-6-phosphatase, PECK) and lipogenesis (e.g., SREBP-1) resulting in decreased hepatic glucose production and TG content. Adiponectin has also been reported to increase glycogen synthesis and aerobic consumption of glucose, and to exert an insulin-mimetic action that has been related to intracellular generation of ROS (58). Exposure of hepatocytes to globular adiponectin leads to a transient generation of ROS through activation of the small GTPase Rac1 and 5-lipoxygenase (5-LOX) (37, 133). This intracellular burst of ROS leads to ligand-independent transactivation of IR through oxidation and inactivation of PTP1B, a phosphotyrosine phosphatase controlling IR phosphorylation. In addition, ROS mediate the downstream response to both globular adiponectin and insulin, including activation of the MAPK cascade and ERK1/2 (58). However, insulin-mediated generation of ROS is related to NADPH-oxidase and not to 5-LOX. This form of redox signaling, based on reversible oxidation of PTPs, represents a strategy adopted by cells to reinforce receptor tyrosine kinase (RTK) signaling by avoiding inactivation by PTPs and has been described to parallel activation of many RTKs (37, 38, 133). These redox-related and insulin-mimetic mechanisms help to understand the in vivo effects of adiponectin on the liver (19, 212) and the muscle (33).

Adipokines, Oxidative Stress, and Inflammation in Fatty Liver

Several studies have addressed the impact of adipokines on the development of hepatocellular damage and inflammation, two major components of steatohepatitis. In most of these studies, a tight relation between adipokine expression and oxidative stress has been found. As circulating levels of adipokines have been shown to have an impact on the development of steatohepatitis, the relationships between oxidative stress and mechanisms of adipokine secretion in the adipose tissue are also briefly discussed.

Leptin

Leptin levels are usually elevated in obese patients, and have been found to correlate with systemic parameters of oxidative stress, suggesting that oxidative stress may contribute to adipokine imbalance in these subjects, especially in the presence of diabetes (164, 198). Leptin, like adiponectin, controls fat catabolism and glucose production activating central neural pathways and increasing hepatic AMPK activity (10, 137). However, the observation that steatosis is associated with elevated leptin levels in obesity indicates the presence of hepatic leptin resistance. The mechanisms of this disturbance are still poorly understood, and a role of nutrients, such as fructose, has recently been suggested (102, 140). Hyperleptinemia in fructose-fed rats is associated with high levels of tyrosine phosphorylation of STAT-3, but not of serine phosphorylation in nuclear STAT-3, suggesting a molecular mechanism for hepatic leptin resistance (140). Another mechanism leading to leptin resistance in this model has been related to increased levels of SOCS-3 and impaired phosphorylation on serine/threonine residues of proteins downstream of the leptin receptor, leading to reduced activation of FOXO1 and AMPK (191). Appearance of leptin resistance has also been linked to the endocannabinoid system, through activation of the CB1 receptor (134). Regulation of leptin receptors may be an additional component contributing to leptin resistance, as in diet-induced obesity, hepatic leptin receptor isoforms were found to be downregulated in the liver (25). Resistance to leptin should explain why obese patients with high leptin have fatty liver despite the anti-steatotic action of this adipokine.

Leptin is involved in innate and adaptive immunity. Survival and cytokine production by T-cells is promoted by leptin, which also stimulates phagocytic activity of macrophages, as well as chemotaxis of polymorphonuclear cells. As a consequence, ob/ob mice show reduced inflammation in autoimmune disease models but are more susceptible toward bacterial or viral infections. Leptin deficiency is associated with increased hepatotoxicity and mortality following endotoxin administration (205), an effect mediated by impaired macrophage function and cytokine imbalance (46). Conversely, leptin-deficient mice show less liver damage in models of T cell-mediated hepatitis, such as that induced by injection of concanavalin A, in association with lower levels of

TNF-α and IL-18 (55). Thus, leptin generally acts as a proinflammatory agent and participates in the protection from microbial infections. Additionally, leptin has a protecting role in models of alcoholic liver damage (13, 181). A possible mechanism underlying this action is the prevention of ethanol-elicited cytotoxicity and apoptosis, which was associated with decreased levels of reactive oxygen species (ROS) and an increase in antioxidant protective pathways (13). Conversely, leptin has also been involved in the generation of oxidative stress. Malondialdehyde levels in the liver of mice treated with a high fat diet were found to be directly related to circulating leptin levels, and leptin expression was upregulated in liver tissue (125).

Adiponectin

Oxidative stress is a relevant step regulating adiponectin secretion at the level of adipose tissue. In general, oxidative stress inhibits adiponectin secretion, linking adipose tissue inflammation, generation of reactive oxygen species, and downregulation of adiponectin secretion (62, 141). These data have been recently extended to children, where obesity-associated oxidative stress was inversely related to the levels of high-molecular weight adiponectin, the more metabolically active complex (11).

The mechanisms leading from adipose tissue expansion in obesity to oxidative stress and reduced adiponectin secretion are complex (Fig. 7). In rats, administration of angiotensin II results in hypertension and endothelial dysfunction, together with a decrease in adiponectin levels and in expression of adiponectin mRNA in the adipose tissue (68). These effects
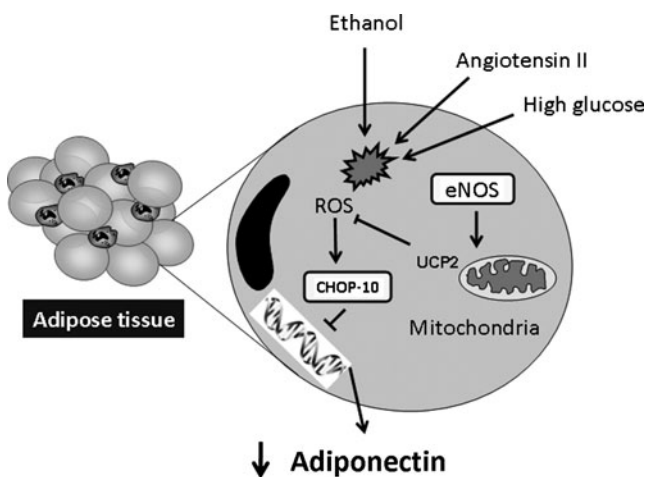

FIG. 7. Mechanisms of reduced adiponectin secretion involving oxidative stress. Generation of reactive oxygen species (ROS) within the adipocyte leads to reduced gene expression and secretion of adiponectin. Increased ROS production may be triggered by ethanol consumption, angiotensin II, or high glucose levels. In contrast, mitochondrial function reduces oxidative stress, partly due to the action of uncoupling protein 2 (UCP2). Reduction of ROS production is favored by eNOS expression, via production of NO, which increases mitochondrial biogenesis. ROS act on adiponectin gene expression by increasing the abundance of CHOP-10, which forms a complex with C/EBPβ, preventing its binding to the adiponectin promoter.

were inhibited by co-treatment with antioxidants, which also reduced angiotensin-mediated upregulation of NADPH oxidase. These results are relevant also to the pathogenesis of steatohepatitis and liver fibrosis, where the renin-angiotensin system plays a pivotal role (15). Interestingly, hydrogen peroxide was found to inhibit adiponectin expression in cultured adipocytes, confirming the negative modulation by oxidative stress (68, 89). The mechanisms underlying inhibition of adiponectin expression by ROS were further investigated in relation to the activity of uncoupling protein-2, which induces adiponectin, while inhibitors of mitochondrial respiration suppress it in a ROS-dependent fashion (36). The inhibition of adiponectin expression by ROS was mediated by upregulation of CHOP-10, which interferes with binding of C/EBPβ to the adiponectin promoter.

High glucose levels may be another factor leading to adipokine imbalance, including reduced adiponectin expression. Exposure of 3T3-L1 adipocytes to constant or intermittent high glucose suppressed the expression of adiponectin, and increased that of resistin in mature adipocytes, compared to normal glucose conditions. These actions were accompanied by increased levels of oxidative stress-related products and nitrotyrosine and were reverted by antioxidants (170). Along these lines, derangements in mitochondrial function have been identified as an additional mechanism relevant to adiponectin expression in the adipose tissue. Adiponectin expression and mitochondrial content in adipose tissue were reduced in obese db/db mice, while in cultured adipocytes, impairment in mitochondrial function decreased adiponectin synthesis (98). More recently, the same group demonstrated that endothelial NO synthase (eNOS) plays an important role in adiponectin synthesis by producing NO and enhancing mitochondrial function in adipocytes (97). Plasma adiponectin concentrations were reduced in eNOS knock-out mice, and this was associated with decreased expression of mitochondrial biogenesis factors, and increased levels of 8-hydroxyguanosine, a biomarker of oxidative stress. NO played a pivotal role, as chronic administration of a NO donor to eNOS-deficient mice increased both plasma adiponectin and adiponectin expression in adipose tissue. Generation of oxidative stress may even overshadow the beneficial effects of weight loss on adiponectin secretion. In subjects with acute weight reduction, increased urinary excretion of molecules related to oxidative stress was accompanied by a decrease in serum adiponectin (203).

Importantly for the pathogenesis of liver injury, ethanol has been shown to reduce the expression of adiponectin in the adipose tissue (34). The decrease in circulating adiponectin caused by chronic alcohol exposure was associated with increased homocysteine, while betaine reduced homocysteine and improved adiponectin (163). Accordingly, supplementation with taurine in a rat model of alcoholic liver injury resulted in normalization of serum levels of adiponectin and of its gene expression at the adipose tissue level, and in reduction of fat accumulation in the liver (35). As a mechanism, taurine prevented the decrease in C/EBP-alpha and PPAR-alpha, which regulate adiponectin expression, in response to ethanol administration. Similarly, treatment with mulberry leaf, which blocks experimental atherosclerosis, was recently shown to increase the expression of adiponectin, and to decrease that of TNF-α, MCP-1, and macrophage markers in white adipose tissue (169). These actions were associated with

reduced expression of NADPH oxidase in both adipose tissue and the liver. In ethanol-induced steatosis, resveratrol, a polyphenol with antioxidant properties, is another factor that limits fat accumulation and leads to increased adiponectin secretion (6). Nonetheless, in some models, adiponectin release may be positively regulated by oxidative stress. For example, downregulation of aldehyde oxidase-1 was associated with reduced adiponectin secretion (194).

Activation of PPAR-γ has emerged as an additional pivotal regulator of adiponectin expression and action. Thiazolidinediones, synthetic PPAR-γ ligands with antidiabetic effects, increase adiponectin levels in humans and induce its expression in experimental models (111). The tissue protective effects of thiazolidinediones are linked to both adiponectin induction and protection from oxidative stress, as exemplified in a model of ischemic heart disease (176). Targeted deletion of PPAR-γ in the adipose tissue, and studies on the adiponectin promoter sequence have confirmed the critical role of this transcription factor in the induction of adiponectin expression (69) (81).

More recently, additional data have indicated that part of the metabolic actions of thiazolidinediones may be mediated through PPAR-γ-dependent modulation of adiponectin receptor expression in different tissues, including the liver. In HepG2 cells, Sun *et al.* showed that rosiglitazone, a thiazolidinedione, increases the mRNA and protein levels of AdipoR2, the predominant adiponectin receptor in hepatocytes (171). These actions were confirmed by *in vivo* studies, where the hepatic levels of AdipoR2 were increased in mice treated with rosiglitazone. Along these lines, modulation of adiponectin receptor expression may have a relevant impact in the pathogenesis of both alcoholic and nonalcoholic steatohepatitis. In a mouse model of alcoholic hepatic steatosis, administration of rosiglitazone resulted in increased circulating levels of adiponectin and upregulated expression of both AdipoR1 and AdipoR2 in the liver (158). Remarkably, these changes were associated with activation of the sirtuin 1–AMPK pathway, and resulted in an amelioration of steatosis. Similarly, in rats administered a high-fat diet, as a model of nonalcoholic fatty liver disease, rosiglitazone improved histology and increased the expression of AdipoR1 and AdipoR2 in liver and visceral fat (109).

Adiponectin has in general a hepatoprotective and antifibrogenic effect in the liver wound healing process. In experimental steatohepatitis, administration of adiponectin ameliorates liver hepatomegaly, steatosis and necro-inflammation, through induction of hepatic fatty acid oxidation and inhibition of fatty acid synthesis (199). Accordingly, in obese mice subjected to damage by galactosamine/LPS, adiponectin administration protected from injury, reducing TNF and increasing PPAR-α (120). In addition, consumption of diet rich in saturated fat, which protects from alcoholic liver damage, increases adiponectin secretion (207). Although adiponectin and leptin share their effects as factors counteracting ectopic fat deposition, they have divergent effects on inflammation. In fact, while leptin-deficient mice are protected from T cell-mediated hepatitis, lipodystrophic mice that lack both adiponectin and leptin are not, unless adiponectin is administered (155). In general, adiponectin reduces inflammation, stimulating secretion of anti-inflammatory cytokines (*e.g.*, IL-10), blocking NF-κB activation, and inhibiting release of TNF-α, IL-6, and chemokines (179). Conversely,

inflammation blocks adiponectin secretion, and specifically, adipose tissue inflammation contributes to reduce plasma adiponectin levels in obesity. Adiponectin protects also against Fas-mediated hepatocyte death (193). Therefore, adiponectin may be envisioned as a negative modulator of systemic and hepatic inflammation that characterizes the metabolic syndrome (66). Nonetheless, hepatic damage triggered by lipotoxicity may occur in spite of increases in adiponectin levels. In mice with steatohepatitis, antisense oligonucleotides interfering with diacylglycerol acyltransferase 2 decreased hepatic steatosis, but increased hepatic free fatty acids, lipid peroxidation, necroinflammation, and fibrosis. Progression of liver damage occurred despite reduced hepatic expression of tumor necrosis factor α and increased serum adiponectin (201). A similar dissociation between steatosis and steatohepatitis has been shown in a recent study where a diet deficient in methionine and choline was administered to mice defective in thioredoxin-binding protein-2 (TBP-2), an endogenous negative regulator of the antioxidant molecule, thioredoxin. These mice showed severe simple steatosis rather than steatohepatitis, and oxidative stress inflammation and hepatic fibrosis were attenuated in TBP-2(-/-) mice (5). These studies clearly indicate that fatty infiltration and steatohepatitis may be dissociated, and only the 'inflammatory' forms may progress to fibrosis.

Adiponectin modulates generation of oxidative stress-related products (Fig. 8). Initial evidence was provided by Yamauchi *et al.*, who cloned the AdipoR1 and R2 receptors and showed their ability to downregulate oxidative stress in the liver and adipose tissue (202). The molecular mechanisms by which this action of adiponectin is exerted are still unclear. In fatty liver, adiponectin downregulated hepatic expression of the enzyme aldehyde oxidase 1, which detoxifies aldehydes and generates oxidative stress (132). This effect was dependent, at least in part, on upregulation of PPAR-α, thus providing a link between the metabolic actions of this adipokine and regulation of the oxidative balance. In skeletal muscle, adiponectin was found to induce a number of NF-κB related genes, including ferritin heavy chain, which together with manganese superoxide dismutase is responsible for the protection from oxidative stress mediated by this adipokine (76). The antioxidant effects of adiponectin have been recently linked also to activation of AdipoR1 and the resulting downstream release of intracellular calcium together with increased activation of AMPK and sirtuin-1 (80). In macrophages, adiponectin modulates the response to lipopolysaccharide dependent on toll-like receptor-4, which leads to TNF-alpha secretion and generation of reactive oxygen species (75). Interestingly, this effect, which is mediated by expression of interleukin-10, occurs after an initial phase during which adiponectin actually increases TNF-α expression, indicating a complex action of adiponectin on the pathways regulating inflammation. Along these lines, in steatotic liver undergoing ischemia-reperfusion, adiponectin siRNA confer protection against oxidative stress and injury (121).

In line with these results, increase in hepatic steatosis and aminotransferase was observed in mice deficient for adiponectin, together with increase in thiobarbituric acid reactive substances, decrease in glutathione levels and expression of antioxidant enzymes (53). It is still unclear to what extent AMPK activation participates in the antioxidant action of
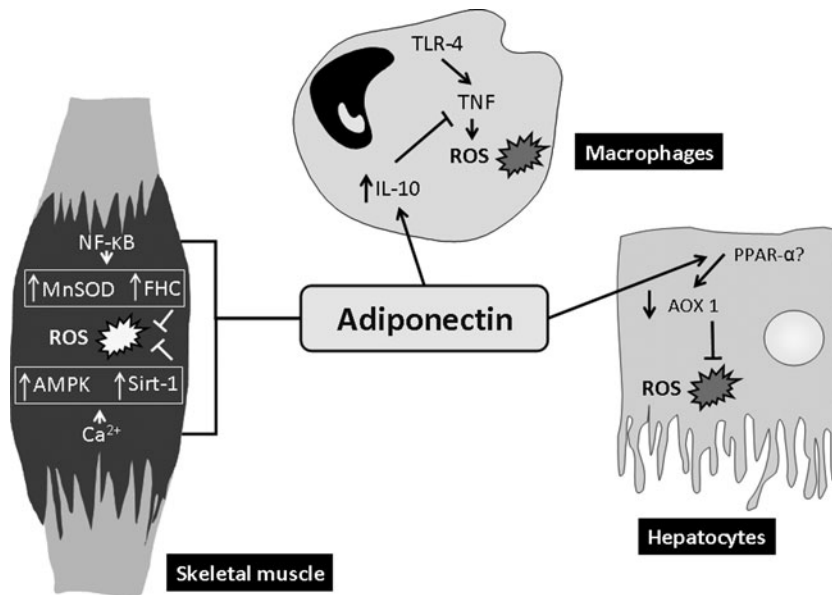
FIG. 8. Adiponectin reduces oxidative stress in different cellular targets. In hepatocytes, binding of adiponectin to its cognate receptors leads to activation of aldehyde oxidase-1 (AOX-1), which reduces intracellular levels of reactive oxygen species (ROS), possibly through increased activation of peroxisome proliferator-activated receptor (PPAR)-α. In macrophages, adiponectin increases the expression of the anti-inflammatory cytokine, interleukin-10 (IL-10). IL-10 blocks the generation of tumor necrosis factor (TNF) in response to activation of toll-like receptor-4 (TLR-4) by lipopolysaccharide. In skeletal muscle, adiponectin activates nuclear factor-κB that results in increased expression of the ferritin heavy chain (FHC) and of manganese superoxide dismutase (MnSOD). In addition, activation of AdipoR1 generates an increase in intracellular calcium that in turn is responsible of activation of AMP-activated protein kinase (AMPK) and of sirtuin-1. All these modifications result in reduced accumulation of ROS.

adiponectin in steatohepatitis. A study recently performed in a model of ischemia-reperfusion myocardial injury suggests that AMPK does indeed participate in protection against ischemia/reperfusion injury, but adiponectin still provides cardioprotection even after interference with AMPK activation (192). More important, treatment of mice defective in AMPK with adiponectin reduced oxidative stress to the same extent as in WT mice. In addition, it has been reported very recently in the same model that in mice made diabetic by administration of a high fat diet, the ability of adiponectin to activate AMPK is blunted, thus providing an additional mechanism for reduced cardioprotection and limitation of oxidative stress in diabetes (206). It still remains to be determined to what extent these data are applicable in the context of steatohepatitis.

Resistin

A limited number of studies have investigated the relevance of resistin in the pathophysiology of NAFLD. Improvement or worsening of hepatic insulin resistance was observed by reducing or increasing resistin levels, respectively (128, 146). The effects on the liver are also mediated centrally, as administration of resistin into the third cerebral ventricle stimulates glucose production independent of circulating levels of glucose-controlling hormones (127). Reduced hepatic glucose production in the absence of resistin is also associated with higher hepatic AMPK activation (14). Hepatic steatosis and VLDL secretion are decreased in resistin-deficient mice placed on a high-fat diet, suggesting a role of resistin in the induction of steatosis (160).

Additional studies link the biology of resistin with hepatic inflammation. In rats, resistin administration significantly worsens inflammation induced by LPS injection (16). In humans, resistin is expressed within the liver during severe damage, including the one associated with NASH, and correlates with infiltration of inflammatory cells (22). In rats, resistin administration significantly worsens inflammation after

LPS injection, through the involvement of the coagulation cascade (16). In addition, in a model of cirrhosis, higher gene and protein expression of resistin and TNF-α was observed in epididymal fat, and TNF injection upregulated resistin (107). Expression of resistin has been documented in quiescent HSC, while activated human HSC respond to resistin with increased expression of pro-inflammatory chemokines and NF-κB activation (22, 157). Resistin expression has been linked to adipose tissue oxidative stress in humans (161). Support to this hypothesis is also provided by the recent observation that uremia increases the expression of resistin and retinol binding protein 4 via increased generation of oxidative stress in the adipose tissue and in isolated adipocytes (42).

Other adipokines

Adipose tissue is a source of a high number of factors, some of which are produced at high levels in other tissues, including the liver. The role of many of these factors in the pathogenesis of insulin resistance and in the development of steatosis and steatohepatitis has been the focus of recent reviews. In light of the limited data on oxidative stress, or for the presence of recent and comprehensive reviews, only a brief mention of the role of these molecules will be made herein.

TNF-α is a well-known pro-inflammatory cytokine that is abundantly released by adipose tissue. In addition to what described in previous sections of the present review, the action of TNF-α is intimately related to the upregulation of intracellular generation of ROS and then of oxidative stress (72, 133, 177–179). Hotamsligil *et al.* linked for the first time TNF-α to obesity, insulin resistance, and chronic inflammation (74), describing described significant elevation of TNF-α in adipose tissue of *db/db* mice. Following that seminal study, several other reports have progressively increased our knowledge on the role of TNF-α in relation to adipose tissue and hepatic NAFLD/NASH. In aggregate, the data summarized below suggest that TNF-α plays important roles in

the progression of NAFLD, including hepatic inflammation and fibrogenesis.

1. Adipose tissue of obese individuals is characterized by increased infiltration of macrophages and so-called "hypertrophied" adipocytes (195), the latter being able to release large quantities of free fatty acids (FFA) via macrophage-induced adipocyte lipolysis. FFA can serve as naturally occurring ligands for Toll-like receptor-4 (167, 168), and FFA increase the production of TNF-α in macrophages through a TLR4/ NFκB pathway, thus establishing a vicious cycle.

2. In the liver, Kupffer cells are the main producer of TNF-α, in a LPS-induced manner. In animal models, activation of Kupffer cells leads to induction of the TNF-α/ TNF receptor signaling pathway, which, as mentioned in the previous section, is critically involved in the pathogenesis of liver fibrosis in NASH (183). Along these lines, *ob/ob* are known to overexpress TNF-α (106) and indeed in these animals, treatment with anti-TNF antibody results in a significant improvement of NAFLD as a consequence of two major events: a) inhibition of c-Jun N-terminal kinase activity (involved in promoting insulin resistance), and b) decreased DNA binding activity of NFκB (involved in the acceleration/ amplification of inflammatory response).

3. In humans, TNF-α levels are significantly increased in both NAFLD and NASH. Moreover, TNF-α levels correlated with hepatic fibrosis in NASH patients (82), with gene expression of both TNF-α and of its receptor being significantly elevated both in hepatic and adipose tissues of human NASH patients (40). Accordingly, serum TNF-α levels have been confirmed to significantly correlate with NAFLD activity score (NAS) (114), a histologic scoring system that is becoming a standard reference in the grading of inflammation and damage in NAFLD. Along these lines, polymorphisms in the TNF-α promoter region and serum level of soluble TNF receptor 2 have been shown to correlate significantly with progression of NAFLD in human patients (180). Finally, the administration of the TNF-α inhibitor pentoxifylline was found to improve amino-transferase serum levels and the insulin resistance index assessed by homeostatic metabolic assessment (HOMA-IR) in NASH patients (2, 145).

Plasminogen activator inhibitors (PAI) decrease fibrinolytic activity by acting as rapid inhibitors of both tissue plasminogen activator (tPA) and urokinase-type plasminogen activator. Both the liver and the adipose tissue are major contributors to production of PAI-1 in humans (54). Insulin, very low-density lipoprotein, and free fatty acids induce PAI-1 production by the liver. In addition, PAI-1 is expressed by HSC and contributes to fibrogenesis (211).

PAI-1 expression in adipose tissue is upregulated in obesity (50), and hyperinsulinemia leads to increased PAI-1 plasma levels and gene expression (142). In addition, modulators of inflammation contribute to upregulation of PAI-1, which is an acute-phase protein. TNF-α, a pro-inflammatory cytokine overexpressed in adipose tissue, is another critical regulator of PAI-1 production (143). Different studies have demonstrated the presence of hypoxia in adipose tissue in conditions of obesity (47), and hypoxia leads to increase

secretion of PAI-1 and decreased adiponectin secretion by adipocytes (70).

Several lines of evidence link generation of oxidative stress and expression of PAI-1 in adipose tissue and different tissues that develop chronic damage (8, 47). In adipocytes, high glucose and advanced glycation end products upregulated PAI-1 expression by oxidative stress-dependent pathways (186). Moreover, oxidative stress and PAI-1 secretion were inhibited by blockers of the renin-angiotensin system, a well-known mediator of adipokine imbalance (101).

In the liver, alcohol-related damage may be prevented, at least in part, by interference with PAI-1 (12). Moreover, upregulation of PAI-1 expression by alcohol in endothelial cells is inhibited by limitation of oxidative stress (162). PAI-1 has also been implicated in fibrogenesis, and in murine embryo fibroblasts the induction of PAI-1 by TGF-β, a profibrogenic cytokine, was mediated by activation of NADPH oxidase 4 and the resulting generation of ROS (108). This pathway causes increased activation of JNK and p38MAPK due to inactivation of MAPK phosphatase 1.

Monocyte chemoattractant protein-1 (MCP-1) is a proinflammatory chemokine that regulates migration of monocytes and lymphocytes. Hepatic expression of MCP-1 is regulated in different conditions of liver injury, and studies in genetically modified animals have identified protective or detrimental roles of this chemokine in various experimental conditions (209). Hepatic MCP-1 expression is also increased in patients with NAFLD, where it correlates with liver fat. This has led to the hypothesis that MCP-1 may be a critical regulator of the changes that are associated with NAFLD and the metabolic syndrome (20). Experimentally, interference with CCR2, the main receptor targeted by MCP-1, reduces metabolic alterations and fatty infiltration of the liver in lipoatrophic mice or in a mouse model of type 2 diabetes (172, 204). At least part of these effects are mediated by recruitment of inflammatory cells to adipose tissue, as indicated by studies in which conditional deletion of MCP-1 in adipose tissue was obtained (90). MCP-1 derived from the adipose tissue has also been shown to directly induce accumulation of lipids in hepatocytes (39).

Several lines of evidence link oxidative stress and MCP-1 expression in adipose tissue. Besides regulating the expression of adiponectin (see above), the antioxidant mulberry leaf inhibits expression of MCP-1 in white adipose tissue (169). In addition, angiotensin receptor blockade was associated with lower MCP-1 expression and generation of oxidative stress (105).

In the liver, a direct link between MCP-1 expression and generation of oxidative stress has been recently demonstrated in an acute model of hepatic damage (209). Hepatic stellate cells are responsible for a significant proportion of MCP-1 expression in the liver, and also in this case oxidative stress has been shown to promote expression of this chemokine (118, 200). Importantly, MCP-1 may be profibrogenic not only through its proinflammatory actions, but also directly, targeting activated stellate cells (119).

In humans, visfatin is increased in association with type 2 diabetes and the metabolic syndrome (137). Recently, visfatin has been identified as a circulating nicotinamide phosphoribosyltransferase, which catalyzes formation of nicotinamide mononucleotide and may influence the function of sirtuins (126). Along these lines, oxidative stress was found to reduce the expression of both sirtuins and visfatin in a monocytic cell

line (44). A role of visfatin in inflammation has also been suggested, and in a group of patients with NAFLD, plasma concentrations of visfatin could predict the presence of portal inflammation (9).

Chemerin regulates glucose uptake in adipocytes and stimulates lipolysis (65), and its serum levels are related to body mass index and the metabolic syndrome (24). In patients with NAFLD, chemerin levels were found to be elevated and to correlate with the NASH activity score (100). Chemerin has been associated with the induction of inflammation (51), and its possible relation with oxidative stress deserves future evaluation.

Adipokines, Oxidative Stress, and HSC Biology

Several *in vitro* and *in vivo* studies demonstrate a pro-fibrogenic role for leptin. Absence of leptin or leptin receptor signaling, as occurring in *ob/ob* mice, and *fa/fa* rats respectively, markedly reduces the development of fibrosis in different experimental models of liver injury, including thioacetamide intoxication, chronic CCl_4 administration, or experimental NASH (78, 104). Whereas deficiency of leptin reduces fibrogenesis, injection of recombinant leptin during acute or chronic liver injury upregulates fibrogenic pathways (77). Conversely, administration of a leptin antagonist, or its co-administration together with recombinant leptin in a model of thioacetamide-induced fibrosis, markedly improves survival (49).

Leptin modulates the biology of different cell types which participate in the liver response to injury, such as Kupffer cells, sinusoidal endothelial cells, and activated HSC. Leptin increases phagocytic activity and cytokine secretion by Kupffer cells and macrophages (46) and stimulates endothelial cells to proliferate and to produce reactive oxygen species (138). Several groups have shown that leptin exerts direct actions on HSC, which express functionally active leptin receptors (7, 149, 151). Leptin modulates genes regulating extracellular matrix deposition and degradation in HSC (31, 149,

173), and in addition, it modulates proliferation and survival, via activation of different intracellular signaling pathways (151), and contributes to amplify the inflammatory response, via NF-κB activation (7). More recently, it has been shown that leptin enhances phagocytosis of apoptotic bodies by HSC (84), an event associated with HSC activation and fibrosis progression (210).

Recent studies have also investigated the intracellular signaling pathways involved in leptin-induced HSC activation and the role of oxidative stress in this context (Fig. 9). As with other cytokine receptors, generation of oxidative stress-related molecules participates in propagation of intracellular signaling. Exposure of LX-2, an immortalized human hepatic stellate cell line, to leptin, increases the intracellular levels of H_2O_2 in a Jak2-mediated fashion, contributing to the upregulation of TIMP-1 and type I procollagen (29, 31). Moreover, leptin was found to repress the basal level of MMP-1 mRNA and its promoter activity, an action again dependent on Jak2-mediated, H_2O_2-dependent, activation of ERK1/2 and p38^{MAPK} (30). An example of the relevance of leptin-induced oxidative stress for the biology of HSC is provided by the effects of curcumin, which was able to block signaling downstream of the ObRb receptor interfering with ROS generation (174).

More recently, De Minicis *et al.* (45) showed that NADPH oxidase is a crucial mediator of the proliferative, fibrogenic, and inflammatory actions of leptin in HSC. Pharmacologic or genetic inhibition of NADPH resulted in reduction of the ability of leptin to induce HSC proliferation and upregulation of fibrogenic and inflammatory molecules (45). In addition, other data provide evidence that exposure of rat HSC to leptin results in the inhibition of the expression and activity of peroxisome proliferator-activated receptor-γ (PPARγ) (213), which maintains HSC quiescence and reverses HSC trans-differentiation to myofibroblasts.

An additional action of leptin that is relevant to the fibrogenic process is the modulation of hepatic angiogenesis. Ac-
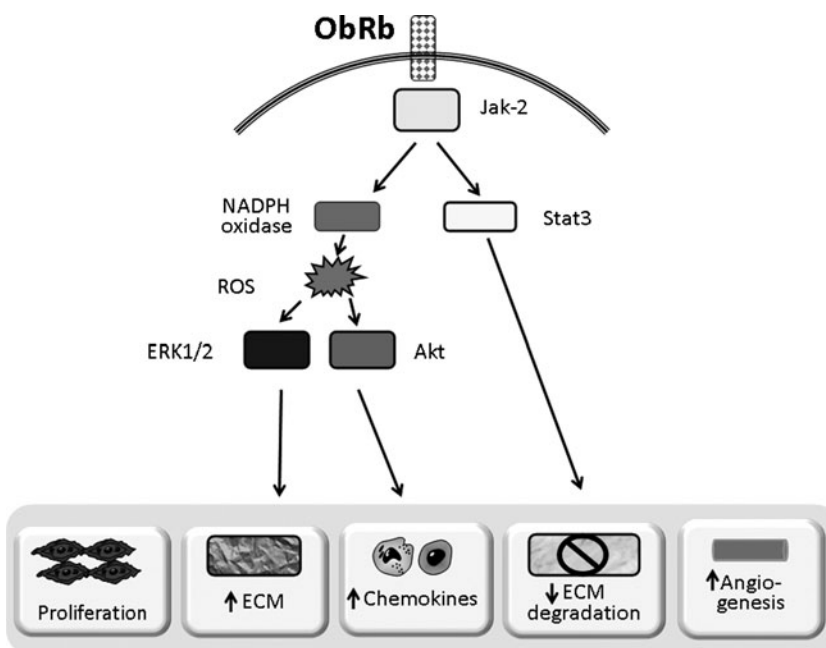

FIG. 9. **Involvement of reactive oxygen species generation in leptin receptor signaling in hepatic stellate cells.** Activation of the 'long form' of leptin receptor, ObRb, results in activation of the nonreceptor tyrosine kinase Jak-2, which activates Stat3 and NADPH oxidase, which leads to ROS generation. ROS contribute to the downstream activation of ERK-1/2 and of Akt. Stat3-dependent and independent signals control a number of biological actions that are pivotal for the actions of HSC in wound healing.

tivation of leptin receptors in HSC leads to upregulated expression of vascular endothelial growth factor, a potent inducer of neovessel formation (7). In addition, leptin exerts a direct angiogenic action on endothelial cells (159). Stimulation of neovessel formation in the liver by leptin is consistent with its profibrogenic role, as angiogenesis is a relevant component of chronic wound healing (190). Recent data indicate the involvement of oxidative stress also in this context (6a). On the other hand, the well-established association between angiogenesis and tumorigenesis suggests a possible additional role of leptin in liver cancer. Along these lines, recent data implicate leptin in the progression of hepatocellular carcinoma (HCC), as leptin increases growth, migration, and invasiveness of HCC cell lines (150). Additionally, leptin stimulates proliferation and metastatic potential of cholangiocarcinoma cells (56). In agreement with these data, lack of leptin is protective in a model of experimental steatohepatitis that leads to development of preneoplastic foci in the liver (96).

The ability of leptin to modulate biological actions through oxidative stress is not limited to the field of hepatic fibrogenesis, but has been involved in the development of atherosclerosis, another condition characterized by altered wound repair. In obese patients, phagocytic NADPH oxidase activity positively correlates with leptin and with carotid intima-media thickness, suggesting that hyperleptinemia may contribute to increased NADPH oxidase activity and early atherosclerosis (60). Leptin infusion in rats results in increased arterial pressure and endothelial dysfunction, two features which are relevant to the metabolic syndrome and to the pathogenesis of atherosclerosis. In aortic and renal tissue, the effects of leptin are mediated by elevation of oxidative stress-related products, including activation of NADPH oxidase (197). In addition, chronic hyperleptinemia reduces the expression of paraoxonase 1, an antioxidant enzyme contained in plasma lipoproteins (18). Of note, reduction of leptin-induced oxidative stress by antioxidant treatment also prevented the decrease in paraoxonase-1 (17). Along these lines, endothelial cells represent an additional target of the proatherogenic action of leptin, where oxidative stress may be involved. In these cells, leptin increased generation of ROS and activated JNK, resulting in increased expression of the proinflammatory chemokine, MCP-1 (23).

A direct antifibrotic effect of adiponectin has been demonstrated in rodents undergoing toxic liver damage, independent of the metabolic actions of this adipokine. Adiponectin knockout mice developed more extensive fibrosis after chronic CCl_4 intoxication, compared to wild-type animals (88). These effects are mediated at least in part by modulation of the activated phenotype of HSC, which express both adiponectin receptors (48). Full-length or globular adiponectin suppress multiple pro-fibrogenic actions of HSC (21), and activation of AMPK has been recently identified as a pivotal mechanism in this context (1, 27). AMPK activation occurs downstream of AdipoR1, but interference with AdipoR2 signaling has been recently shown to be sufficient to block the progression of experimental steatohepatitis (182). Adiponectin knockout mice develop more extensive fibrosis than wild-type animals after chronic CCl_4 intoxication, demonstrating that adiponectin has antifibrogenic effects independently of metabolic actions (88).

Appearance of hepatocellular carcinoma is a well-established consequence of fibrogenic liver diseases, and additional information has recently become available on the role of adiponectin in liver cancer. Administration of a choline-deficient, amino acid-defined diet to adiponectin-deficient mice resulted in increased incidence of liver tumors, together with increased levels of oxidative stress (87). In addition, lack of adiponectin was found to delay liver regeneration (53). In an orthotopic liver tumor model in nude mice, injection of adenovirus encoding adiponectin inhibited tumor growth, and was associated with lower appearance of distant metastases (113). These effects were accompanied by reduced activation of hepatic stellate cells and angiogenesis. Very recently, Saxena *et al*. (148) have shown that in hepatocellular carcinoma cells adiponectin increases apoptosis, inducing JNK phosphorylation. In addition, adiponectin-mediated increase in AMPK phosphorylation is a critical event for the induction of JNK phosphorylation and the biological effects of adiponectin. In parallel, knockout of JNK1 was found to protect from experimental steatohepatitis and to result in elevated adiponectin levels, thus demonstrating the complex role played by this mitogen-activated protein kinase in the context of adipokine biology and the pathogenesis of steatohepatitis (152).

Perspectives

The field of adipokines has witnessed a tremendous expansion in the last 5 years, and has led to the recognition of the role of adipose tissue and its products in several chronic diseases, including those affecting the liver. In addition, evidence for a cross-talk between adipokine biology, inflammation, oxidative stress, and tissue repair has been provided in several tissues in conditions of chronic damage.

In spite of the data that have accumulated, several points still await further clarification with experimental studies. In particular, the dual role of reactive oxygen species as intracellular signaling molecules and products accumulating during injury, make it difficult to reach general conclusions. Areas that will need additional investigation include: (a) elucidation of the role of oxidative stress in the context of chronic inflammation as a mediator of insulin resistance, as opposed to its action in the signaling cascade downstream of insulin receptor activation; (b) evaluation of the reasons why an approach with antioxidants, that is successful in animal models, has been still poorly validated in humans with insulin resistance and/or fatty liver; (c) detailed investigation of the role played by oxidative stress in mediating the response of the liver to adipokines, especially in the context of the angiogenic role of leptin and the anti-inflammatory and hepatoprotective role of adiponectin. As numerous laboratories are focused on the significance of this intriguing group of molecules, novel data are likely to be available very soon.

Acknowledgments

References

1. Adachi M and Brenner DA. High molecular weight adiponectin inhibits proliferation of hepatic stellate cells via activation of adenosine monophosphate-activated protein kinase. *Hepatology* 47: 677–685, 2008.

2. Adams LA, Zein CO, Angulo P, and Lindor KD. A pilot trial of pentoxifylline in nonalcoholic steatohepatitis. *Am J Gastroenterol* 99: 2365–2368, 2004.

3. Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, and Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *Hepatology* 33: 1358–1364, 2001.

4. Aguirre V, Uchida T, Yenush L, Davis R, and White MF. The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). *J Biol Chem* 275: 9047–9054, 2000.

5. Ahsan MK, Okuyama H, Hoshino Y, Oka S, Masutani H, Yodoi J, and Nakamura H. Thioredoxin-binding protein-2 deficiency enhances methionine-choline deficient diet-induced hepatic steatosis but inhibits steatohepatitis in mice. *Antioxid Redox Signal* 11: 2573–2584, 2009.

6. Ajmo JM, Liang X, Rogers CQ, Pennock B, and You M. Resveratrol alleviates alcoholic fatty liver in mice. *Am J Physiol -Gastroint Liver Physiol* 295: G833–842, 2008.

6a. Aleffi S, Navari N, Delogu W, Galastri S, Novo E, Rombouts K, Pinzani M, Parola M, and Marra F. The mammalian target of rapamycin mediates the angiogenic effects of leptin in human hepatic stellate cells. *Am J Physiol Gastrointest Liver Physiol* 2011. [Epub ahead of print].

7. Aleffi S, Petrai I, Bertolani C, Parola M, Colombatto S, Novo E, Vizzutti F, Anania FA, Milani S, Rombouts K, Laffi G, Pinzani M, and Marra F. Upregulation of proinflammatory and proangiogenic cytokines by leptin in human hepatic stellate cells. *Hepatology* 42: 1339–1348., 2005.

8. Alessi MC, Poggi M, and Juhan–Vague I. Plasminogen activator inhibitor-1, adipose tissue and insulin resistance. *Curr Opin Lipidol* 18: 240–245, 2007.

9. Aller R, de Luis DA, Izaola O, Sagrado MG, Conde R, Velasco MC, Alvarez T, Pacheco D, and Gonzalez JM. Influence of visfatin on histopathological changes of non-alcoholic fatty liver disease. *Dig Dis Sci* 54: 1772–1777, 2009.

10. Andreelli F, Foretz M, Knauf C, Cani PD, Perrin C, Iglesias MA, Pillot B, Bado A, Tronche F, Mithieux G, Vaulont S, Burcelin R, and Viollet B. Liver adenosine monophosphate-activated kinase-alpha2 catalytic subunit is a key target for the control of hepatic glucose production by adiponectin and leptin but not insulin. *Endocrinology* 147: 2432–2441, 2006.

11. Araki S, Dobashi K, Yamamoto Y, Asayama K, and Kusuhara K. Increased plasma isoprostane is associated with visceral fat, high molecular weight adiponectin, and metabolic complications in obese children. *Eur J Pediatr* 169: 965–970, 2010.

12. Arteel GE. New role of plasminogen activator inhibitor-1 in alcohol-induced liver injury. *J Gastroenterol Hepatol* 23 Suppl 1: S54–59, 2008.

13. Balasubramaniyan V, Murugaiyan G, Shukla R, Bhonde RR, and Nalini N. Leptin downregulates ethanol-induced secretion of proinflammatory cytokines and growth factor. *Cytokine* 37: 96–100, 2007.

14. Banerjee RR, Rangwala SM, Shapiro JS, Rich AS, Rhoades B, Qi Y, Wang J, Rajala MW, Pocai A, Scherer PE, Steppan CM, Ahima RS, Obici S, Rossetti L, and Lazar MA. Regulation of fasted blood glucose by resistin. *Science* 303: 1195–1198, 2004.

15. Bataller R and Brenner D. Liver fibrosis. *J Clin Invest* 115: 209–218, 2005.

16. Beier JI, Guo L, von Montfort C, Kaiser JP, Joshi-Barve S, and Arteel GE. New role of resistin in lipopolysaccharide-induced liver damage in mice. *J Pharmacol Exp Ther* 325: 801–808, 2008.

17. Beltowski J, Jamroz–Wisniewska A, Borkowska E, and Wojcicka G. Differential effect of antioxidant treatment on plasma and tissue paraoxonase activity in hyperleptinemic rats. *Pharmacol Res* 51: 523–532, 2005.

18. Beltowski J, Wojcicka G, and Jamroz A. Leptin decreases plasma paraoxonase 1 (PON1) activity and induces oxidative stress: the possible novel mechanism for proatherogenic effect of chronic hyperleptinemia. *Atherosclerosis* 170: 21–29, 2003.

19. Berg AH, Combs TP, and Scherer PE. ACRP30/adiponectin: An adipokine regulating glucose and lipid metabolism. *Trends Endocrin Met* 13: 84–89, 2002.

20. Berres ML, Nellen A, and Wasmuth HE. Chemokines as immune mediators of liver diseases related to the metabolic syndrome. *Dig Dis* 28: 192–196, 2010.

21. Bertolani C and Marra F. Role of adipocytokines in hepatic fibrosis. *Curr Pharm Design* 16: 1929–1940, 2010.

22. Bertolani C, Sancho–Bru P, Failli P, Bataller R, Aleffi S, DeFranco R, Mazzinghi B, Romagnani P, Milani S, Gines P, Colmenero J, Parola M, Gelmini S, Tarquini R, Laffi G, Pinzani M, and Marra F. Resistin as an intrahepatic cytokine: Overexpression during chronic injury and induction of proinflammatory actions in hepatic stellate cells. *Am J Pathol* 169: 2042–2053, 2006.

23. Bouloumie A, Marumo T, Lafontan M, and Busse R. Leptin induces oxidative stress in human endothelial cells. *FASEB J* 13: 1231–1238, 1999.

24. Bozaoglu K, Bolton K, McMillan J, Zimmet P, Jowett J, Collier G, Walder K, and Segal D. Chemerin is a novel adipokine associated with obesity and metabolic syndrome. *Endocrinology* 148: 4687–4694, 2007.

25. Brabant G, Muller G, Horn R, Anderwald C, Roden M, and Nave H. Hepatic leptin signaling in obesity. *FASEB J* 19: 1048–1050, 2005.

26. Bugianesi E, Gastaldelli A, Vanni E, Gambino R, Cassader M, Baldi S, Ponti V, Pagano G, Ferrannini E, and Rizzetto M. Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: Sites and mechanisms. *Diabetologia* 48: 634–642, 2005.

27. Caligiuri A, Bertolani C, Guerra CT, Aleffi S, Galastri S, Trappoliere M, Vizzutti F, Gelmini S, Laffi G, Pinzani M, and Marra F. Adenosine monophosphate-activated protein kinase modulates the activated phenotype of hepatic stellate cells. *Hepatology* 47: 668–676, 2008.

28. Calle EE, Rodriguez C, Walker–Thurmond K, and Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 348: 1625–1638, 2003.

29. Cao Q, Mak KM, and Lieber CS. Leptin enhances alpha1(I) collagen gene expression in LX-2 human hepatic stellate cells through JAK-mediated H2O2-dependent MAPK pathways. *J Cell Biochem* 97: 188–197, 2006.

30. Cao Q, Mak KM, and Lieber CS. Leptin represses matrix metalloproteinase-1 gene expression in LX2 human hepatic stellate cells. *J Hepatol* 46: 124–133, 2007.

31. Cao Q, Mak KM, Ren C, and Lieber CS. Leptin stimulates tissue inhibitor of metalloproteinase-1 in human hepatic stellate cells: Respective roles of the JAK/STAT and JAK-mediated H2O2-dependant MAPK pathways. *J Biol Chem* 279: 4292–4304, 2004.

32. Capeau J. Insulin resistance and steatosis in humans. *Diabetes Metab* 34: 649–657, 2008.

33. Ceddia RB, Somwar R, Maida A, Fang X, Bikopoulos G, and Sweeney G. Globular adiponectin increases GLUT4 translocation and glucose uptake but reduces glycogen synthesis in rat skeletal muscle cells. *Diabetologia* 48: 132–139, 2005.

34. Chen X, Sebastian BM, and Nagy LE. Chronic ethanol feeding to rats decreases adiponectin secretion by subcutaneous adipocytes. *Am J Physiol Endocrinol Metab* 292: E621–628, 2007.

35. Chen X, Sebastian BM, Tang H, McMullen MM, Axhemi A, Jacobsen DW, and Nagy LE. Taurine supplementation prevents ethanol-induced decrease in serum adiponectin and reduces hepatic steatosis in rats. *Hepatology* 49: 1554–1562, 2009.

36. Chevillotte E, Giralt M, Miroux B, Ricquier D, and Villarroya F. Uncoupling protein-2 controls adiponectin gene expression in adipose tissue through the modulation of reactive oxygen species production. *Diabetes* 56: 1042–1050, 2007.

37. Chiarugi P and Cirri P. Redox regulation of protein tyrosine phosphatases during receptor tyrosine kinase signal transduction. *Trends Biochem Sci* 28: 509–514, 2003.

38. Chiarugi P and Fiaschi T. Adiponectin in health and diseases: From metabolic syndrome to tissue regeneration. *Expert Opin Ther Targets* 14: 193–206, 2010.

39. Clement S, Juge–Aubry C, Sgroi A, Conzelmann S, Pazienza V, Pittet–Cuenod B, Meier CA, and Negro F. Monocyte chemoattractant protein-1 secreted by adipose tissue induces direct lipid accumulation in hepatocytes. *Hepatology* 48: 799–807, 2008.

40. Crespo J, Cayon A, Fernandez–Gil P, Hernandez–Guerra M, Mayorga M, Dominguez–Diez A, Fernandez–Escalante JC, and Pons–Romero F. Gene expression of tumor necrosis factor alpha and TNF-receptors, p55 and p75, in nonalcoholic steatohepatitis patients. *Hepatology* 34: 1158–1163, 2001.

41. Cubero FJ, Drvarov O, and Trautwein C. c-Jun NH(2)-terminal kinase 1 in hepatocytes: An essential mediator of insulin resistance. *Hepatology* 51: 2221–2223, 2010.

42. D'Apolito M, Du X, Zong H, Catucci A, Maiuri L, Trivisano T, Pettoello–Mantovani M, Campanozzi A, Raia V, Pessin JE, Brownlee M, and Giardino I. Urea-induced ROS generation causes insulin resistance in mice with chronic renal failure. *J Clin Invest* 120: 203–213, 2010.

43. Day CP. From fat to inflammation. *Gastroenterology* 130: 207–210, 2006.

44. de Kreutzenberg SV, Ceolotto G, Papparella I, Bortoluzzi A, Semplicini A, Dalla Man C, Cobelli C, Fadini GP, and Avogaro A. Downregulation of the longevity-associated protein sirtuin 1 in insulin resistance and metabolic syndrome: Potential biochemical mechanisms. *Diabetes* 59: 1006–1015, 2010.

45. De Minicis S, Candelaresi C, Marzioni M, Saccomano S, Roskams T, Casini A, Risaliti A, Salzano R, Cautero N, di Francesco F, Benedetti A, and Svegliati–Baroni G. Role of endogenous opioids in modulating HSC activity *in vitro* and liver fibrosis *in vivo*. *Gut* 57: 352–364, 2008.

46. Diehl AM. Nonalcoholic steatosis and steatohepatitis IV. Nonalcoholic fatty liver disease abnormalities in macrophage function and cytokines. *Am J Physiol Gastroint Liver Physiol* 282: G1–5, 2002.

47. Dimova EY, Samoylenko A, and Kietzmann T. Oxidative stress and hypoxia: Implications for plasminogen activator inhibitor-1 expression. *Antioxid Redox Signal* 6: 777–791, 2004.

48. Ding X, Saxena NK, Lin S, Xu A, Srinivasan S, and Anania FA. The roles of leptin and adiponectin: A novel paradigm in adipocytokine regulation of liver fibrosis and stellate cell biology. *Am J Pathol* 166: 1655–1669, 2005.

49. Elinav E, Pappo O, Sklair–Levy M, Margalit M, Shibolet O, Gomori M, Alper R, Thalenfeld B, Engelhardt D, Rabbani E, and Ilan Y. Adoptive transfer of regulatory NKT lymphocytes ameliorates non-alcoholic steatohepatitis and glucose intolerance in ob/ob mice and is associated with intrahepatic CD8 trapping. *J Pathol* 209: 121–128, 2006.

50. Eriksson P, Reynisdottir S, Lonnqvist F, Stemme V, Hamsten A, and Arner P. Adipose tissue secretion of plasminogen activator inhibitor-1 in non-obese and obese individuals. *Diabetologia* 41: 65–71, 1998.

51. Ernst MC and Sinal CJ. Chemerin: At the crossroads of inflammation and obesity. *Trends Endocrin Met* 21: 660–667, 2010.

52. Evans JL, Maddux BA, and Goldfine ID. The molecular basis for oxidative stress-induced insulin resistance. *Antioxid Redox Signal* 7: 1040–1052, 2005.

53. Ezaki H, Yoshida Y, Saji Y, Takemura T, Fukushima J, Matsumoto H, Kamada Y, Wada A, Igura T, Kihara S, Funahashi T, Shimomura I, Tamura S, Kiso S, and Hayashi N. Delayed liver regeneration after partial hepatectomy in adiponectin knockout mice. *Biochem Biophy Rese Commun* 378: 68–72, 2009.

54. Faber DR, de Groot PG, and Visseren FL. Role of adipose tissue in haemostasis, coagulation and fibrinolysis. *Obes Rev* 10: 554–563, 2009.

55. Faggioni R, Jones–Carson J, Reed DA, Dinarello CA, Feingold KR, Grunfeld C, and Fantuzzi G. Leptin-deficient (ob/ob) mice are protected from T cell-mediated hepatotoxicity: role of tumor necrosis factor alpha and IL-18. *Proc Natl Acad Sci USA* 97: 2367–2372, 2000.

56. Fava G, Alpini G, Rychlicki C, Saccomanno S, DeMorrow S, Trozzi L, Candelaresi C, Venter J, Di Sario A, Marzioni M, Bearzi I, Glaser S, Alvaro D, Marucci L, Francis H, Svegliati–Baroni G, and Benedetti A. Leptin enhances cholangiocarcinoma cell growth. *Cancer Res* 68: 6752–6761, 2008.

57. Ferre P and Foufelle F. SREBP-1c transcription factor and lipid homeostasis: Clinical perspective. *Horm Res* 68: 72–82, 2007.

58. Fiaschi T, Buricchi F, Cozzi G, Matthias S, Parri M, Raugei G, Ramponi G, and Chiarugi P. Redox-dependent and ligand-independent trans-activation of insulin receptor by globular adiponectin. *Hepatology* 46: 130–139, 2007.

59. Fisher SJ and Kahn CR. Insulin signaling is required for insulin's direct and indirect action on hepatic glucose production. *J Clin Invest* 111: 463–468, 2003.

60. Fortuno A, Bidegain J, Baltanas A, Moreno MU, Montero L, Landecho MF, Beloqui O, Diez J, and Zalba G. Is leptin involved in phagocytic NADPH oxidase overactivity in obesity? Potential clinical implications. *J Hypertens* 28: 1944–1950, 2010.

61. Frescas D, Valenti L, and Accili D. Nuclear trapping of the forkhead transcription factor FoxO1 via Sirt-dependent deacetylation promotes expression of glucogenetic genes. *J Biol Chem* 280: 20589–20595, 2005.

62. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, and Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 114: 1752–1761, 2004.

63. Gao Z, Hwang D, Bataille F, Lefevre M, York D, Quon MJ, and Ye J. Serine phosphorylation of insulin receptor substrate 1 by inhibitor kappa B kinase complex. *J Biol Chem* 277: 48115–48121, 2002.

64. Ginsberg HN, Zhang YL, and Hernandez–Ono A. Regulation of plasma triglycerides in insulin resistance and diabetes. *Arch Med Res* 36: 232–240, 2005.

65. Goralski KB, McCarthy TC, Hanniman EA, Zabel BA, Butcher EC, Parlee SD, Muruganandan S, and Sinal CJ. Chemerin, a novel adipokine that regulates adipogenesis and adipocyte metabolism. *J Biol Chem* 282: 28175–28188, 2007.

66. Gustafson B, Hammarstedt A, Andersson CX, and Smith U. Inflamed adipose tissue: A culprit underlying the metabolic syndrome and atherosclerosis. *Arterioscler Thromb Vasc Biol* 27: 2276–2283, 2007.

67. Haeusler RA and Accili D. The double life of Irs. *Cell Metabolism* 8: 7–9, 2008.

68. Hattori Y, Akimoto K, Gross SS, Hattori S, and Kasai K. Angiotensin-II-induced oxidative stress elicits hypoadiponectinaemia in rats. *Diabetologia* 48: 1066–1074, 2005.

69. He W, Barak Y, Hevener A, Olson P, Liao D, Le J, Nelson M, Ong E, Olefsky JM, and Evans RM. Adipose-specific peroxisome proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle. *Proc Natl Acad Sci USA* 100: 15712–15717, 2003.

70. Hosogai N, Fukuhara A, Oshima K, Miyata Y, Tanaka S, Segawa K, Furukawa S, Tochino Y, Komuro R, Matsuda M, and Shimomura I. Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. *Diabetes* 56: 901–911, 2007.

71. Hotamisligil GS. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell* 140: 900–917, 2010.

72. Hotamisligil GS. Inflammation and metabolic disorders. *Nature* 444: 860–867, 2006.

73. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, and Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* 271: 665–668, 1996.

74. Hotamisligil GS, Shargill NS, and Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: Direct role in obesity-linked insulin resistance. *Science* 259: 87–91., 1993.

75. Huang H, Park PH, McMullen MR, and Nagy LE. Mechanisms for the anti-inflammatory effects of adiponectin in macrophages. *J Gastroenterol Hepatol* 23 Suppl 1: S50–53, 2008.

76. Ikegami Y, Inukai K, Imai K, Sakamoto Y, Katagiri H, Kurihara S, Awata T, and Katayama S. Adiponectin upregulates ferritin heavy chain in skeletal muscle cells. *Diabetes* 58: 61–70, 2009.

77. Ikejima K, Honda H, Yoshikawa M, Hirose M, Kitamura T, Takei Y, and Sato N. Leptin augments inflammatory and profibrogenic responses in the murine liver induced by hepatotoxic chemicals. *Hepatology* 34: 288–297, 2001.

78. Ikejima K, Takei Y, Honda H, Hirose M, Yoshikawa M, Zhang YJ, Lang T, Fukuda T, Yamashina S, Kitamura T, and Sato N. Leptin receptor-mediated signaling regulates hepatic fibrogenesis and remodeling of extracellular matrix in the rat. *Gastroenterology* 122: 1399–1410, 2002.

79. Inoguchi T, Li P, Umeda F, Yu HY, Kakimoto M, Imamura M, Aoki T, Etoh T, Hashimoto T, Naruse M, Sano H, Utsumi H, and Nawata H. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C–dependent activation of NAD(P)H oxidase in cultured vascular cells. *Diabetes* 49: 1939–1945, 2000.

80. Iwabu M, Yamauchi T, Okada–wabu M, Sato K, Nakagawa T, Funata M, Yamaguchi M, Namiki S, Nakayama R, Tabata M, Ogata H, Kubota N, Takamoto I, Hayashi YK, Yamauchi N, Waki H, Fukayama M, Nishino I, Tokuyama K, Ueki K, Oike Y, Ishii S, Hirose K, Shimizu T, Touhara K, and Kadowaki T. Adiponectin and AdipoR1 regulate PGC-1alpha and mitochondria by Ca(2+) and AMPK/SIRT1. *Nature* 464: 1313–1319, 2010.

81. Iwaki M, Matsuda M, Maeda N, Funahashi T, Matsuzawa Y, Makishima M, and Shimomura I. Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. *Diabetes* 52: 1655–1663, 2003.

82. Jarrar MH, Baranova A, Collantes R, Ranard B, Stepanova M, Bennett C, Fang Y, Elariny H, Goodman Z, Chandhoke V, and Younossi ZM. Adipokines and cytokines in non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 27: 412–421, 2008.

83. Ji C. Dissection of endoplasmic reticulum stress signaling in alcoholic and non-alcoholic liver injury. *J Gastroenterol Hepatol* 23 Suppl 1: S16–24, 2008.

84. Jiang JX, Mikami K, Shah VH, and Torok NJ. Leptin induces phagocytosis of apoptotic bodies by hepatic stellate cells via a Rho guanosine triphosphatase-dependent mechanism. *Hepatology* 48: 1497–1505, 2008.

85. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, and Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest* 116: 1784–1792, 2006.

86. Kahn CR. Banting Lecture. Insulin action, diabetogenes, and the cause of type II diabetes. *Diabetes* 43: 1066–1084, 1994.

87. Kamada Y, Matsumoto H, Tamura S, Fukushima J, Kiso S, Fukui K, Igura T, Maeda N, Kihara S, Funahashi T, Matsuzawa Y, Shimomura I, and Hayashi N. Hypoadiponectinemia accelerates hepatic tumor formation in a nonalcoholic steatohepatitis mouse model. *J Hepatol* 47: 556–564, 2007.

88. Kamada Y, Tamura S, Kiso S, Matsumoto H, Saji Y, Yoshida Y, Fukui K, Maeda N, Nishizawa H, Nagaretani H, Okamoto Y, Kihara S, Miyagawa J, Shinomura Y, Funahashi T, and Matsuzawa Y. Enhanced carbon tetrachloride-induced liver fibrosis in mice lacking adiponectin. *Gastroenterology* 125: 1796–1807, 2003.

89. Kamigaki M, Sakaue S, Tsujino I, Ohira H, Ikeda D, Itoh N, Ishimaru S, Ohtsuka Y, and Nishimura M. Oxidative stress provokes atherogenic changes in adipokine gene expression in 3T3-L1 adipocytes. *Biochem Biophys Res Commun* 339: 624–632, 2006.

90. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K–I, Kitazawa R, Kitazawa S, Miyachi H, Maeda S, Egashira K, and Kasuga M. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest* 116: 1494–1505, 2006.

91. Kawaguchi T, Yoshida T, Harada M, Hisamoto T, Nagao Y, Ide T, Taniguchi E, Kumemura H, Hanada S, Maeyama M, Baba S, Koga H, Kumashiro R, Ueno T, Ogata H, Yoshimura A, and Sata M. Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of

suppressor of cytokine signaling 3. *Am J Pathol* 165: 1499–1508, 2004.

92. Kim HJ, Lee KE, Kim DJ, Kim SK, Ahn CW, Lim SK, Kim KR, Lee HC, Huh KB, and Cha BS. Metabolic significance of nonalcoholic fatty liver disease in nonobese, nondiabetic adults. *Arch Intern Med* 164: 2169–2175, 2004.

93. Kim JK, Fillmore JJ, Chen Y, Yu C, Moore IK, Pypaert M, Lutz EP, Kako Y, Velez–Carrasco W, Goldberg IJ, Breslow JL, and Shulman GI. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. *Proc Natl Acad Sci USA* 98: 7522–7527, 2001.

94. Kim JK, Kim YJ, Fillmore JJ, Chen Y, Moore I, Lee J, Yuan M, Li ZW, Karin M, Perret P, Shoelson SE, and Shulman GI. Prevention of fat-induced insulin resistance by salicylate. *J Clin Invest* 108: 437–446, 2001.

95. Kim SP, Ellmerer M, Van Citters GW, and Bergman RN. Primacy of hepatic insulin resistance in the development of the metabolic syndrome induced by an isocaloric moderate-fat diet in the dog. *Diabetes* 52: 2453–2460, 2003.

96. Kitade M, Yoshiji H, Kojima H, Ikenaka Y, Noguchi R, Kaji K, Yoshii J, Yanase K, Namisaki T, Asada K, Yamazaki M, Tsujimoto T, Akahane T, Uemura M, and Fukui H. Leptin-mediated neovascularization is a prerequisite for progression of nonalcoholic steatohepatitis in rats. *Hepatology* 44: 983–991, 2006.

97. Koh EH, Kim M, Ranjan KC, Kim HS, Park HS, Oh KS, Park IS, Lee WJ, Kim MS, Park JY, Youn JH, and Lee KU. eNOS plays a major role in adiponectin synthesis in adipocytes. *Am J Physiol Endocrinol Metab* 298: E846–853, 2010.

98. Koh EH, Park JY, Park HS, Jeon MJ, Ryu JW, Kim M, Kim SY, Kim MS, Kim SW, Park IS, Youn JH, and Lee KU. Essential role of mitochondrial function in adiponectin synthesis in adipocytes. *Diabetes* 56: 2973–2981, 2007.

99. Kubota N, Kubota T, Itoh S, Kumagai H, Kozono H, Takamoto I, Mineyama T, Ogata H, Tokuyama K, Ohsugi M, Sasako T, Moroi M, Sugi K, Kakuta S, Iwakura Y, Noda T, Ohnishi S, Nagai R, Tobe K, Terauchi Y, Ueki K, and Kadowaki T. Dynamic functional relay between insulin receptor substrate 1 and 2 in hepatic insulin signaling during fasting and feeding. *Cell Metabolism* 8: 49–64, 2008.

100. Kukla M, Zwirska–Korczala K, Hartleb M, Waluga M, Chwist A, Kajor M, Ciupinska–Kajor M, Berdowska A, Wozniak–Grygiel E, and Buldak R. Serum chemerin and vaspin in non-alcoholic fatty liver disease. *Scand J Gastroenterol* 45: 235–242, 2010.

101. Kurata A, Nishizawa H, Kihara S, Maeda N, Sonoda M, Okada T, Ohashi K, Hibuse T, Fujita K, Yasui A, Hiuge A, Kumada M, Kuriyama H, Shimomura I, and Funahashi T. Blockade of angiotensin II type-1 receptor reduces oxidative stress in adipose tissue and ameliorates adipocytokine dysregulation. *Kidney Int* 70: 1717–1724, 2006.

102. Le KA, Faeh D, Stettler R, Ith M, Kreis R, Vermathen P, Boesch C, Ravussin E, and Tappy L. A 4-wk high-fructose diet alters lipid metabolism without affecting insulin sensitivity or ectopic lipids in healthy humans. *Am J Clin Nutr* 84: 1374–1379, 2006.

103. Leclercq IA, Da Silva Morais A, Schroyen B, Van Hul N, and Geerts A. Insulin resistance in hepatocytes and sinusoidal liver cells: mechanisms and consequences. *J Hepatol* 47: 142–156, 2007.

104. Leclercq IA, Farrell GC, Schriemer R, and Robertson GR. Leptin is essential for the hepatic fibrogenic response to chronic liver injury. *J Hepatol* 37: 206–213, 2002.

105. Lee MH, Song HK, Ko GJ, Kang YS, Han SY, Han KH, Kim HK, Han JY, and Cha DR. Angiotensin receptor blockers improve insulin resistance in type 2 diabetic rats by modulating adipose tissue. *Kidney Int* 74: 890–900, 2008.

106. Li Z, Yang S, Lin H, Huang J, Watkins PA, Moser AB, Desimone C, Song XY, and Diehl AM. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. *Hepatology* 37: 343–350, 2003.

107. Lin SY, Sheu WH, Chen WY, Lee FY, and Huang CJ. Stimulated resistin expression in white adipose of rats with bile duct ligation-induced liver cirrhosis: relationship to cirrhotic hyperinsulinemia and increased tumor necrosis factor-alpha. *Mol Cell Endocrinol* 232: 1–8, 2005.

108. Liu RM, Choi J, Wu JH, Gaston Pravia KA, Lewis KM, Brand JD, Mochel NS, Krzywanski DM, Lambeth JD, Hagood JS, Forman HJ, Thannickal VJ, and Postlethwait EM. Oxidative modification of nuclear mitogen-activated protein kinase phosphatase 1 is involved in transforming growth factor beta1-induced expression of plasminogen activator inhibitor 1 in fibroblasts. *J Biol Chem* 285: 16239–16247, 2010.

109. Liu S, Wu HJ, Zhang ZQ, Chen Q, Liu B, Wu JP, and Zhu L. The ameliorating effect of rosiglitazone on experimental nonalcoholic steatohepatitis is associated with regulating adiponectin receptor expression in rats. *Eur J Pharmacol* 650: 384–389, 2011.

110. Luo J, Field SJ, Lee JY, Engelman JA, and Cantley LC. The p85 regulatory subunit of phosphoinositide 3-kinase downregulates IRS-1 signaling via the formation of a sequestration complex. *J Cell Biol* 170: 455–464, 2005.

111. Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, Ouchi N, Kuriyama H, Hotta K, Nakamura T, Shimomura I, and Matsuzawa Y. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes* 50: 2094–2099, 2001.

112. Mahadev K, Motoshima H, Wu X, Ruddy JM, Arnold RS, Cheng G, Lambeth JD, and Goldstein BJ. The NAD(P)H oxidase homolog Nox4 modulates insulin-stimulated generation of H2O2 and plays an integral role in insulin signal transduction. *Mol Cell Biol* 24: 1844–1854, 2004.

113. Man K, Ng KTP, Xu A, Cheng Q, Lo CM, Xiao JW, Sun BS, Lim ZXH, Cheung JS, Wu EX, Sun CKW, Poon RTP, and Fan ST. Suppression of liver tumor growth and metastasis by adiponectin in nude mice through inhibition of tumor angiogenesis and downregulation of Rho kinase/IFN-inducible protein 10/matrix metalloproteinase 9 signaling. *Clin Cancer Res* 16: 967–977, 2010.

114. Manco M, Marcellini M, Giannone G, and Nobili V. Correlation of serum TNF-alpha levels and histologic liver injury scores in pediatric nonalcoholic fatty liver disease. *Am J Clin Pathol* 127: 954–960, 2007.

115. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, and Melchionda N. Nonalcoholic fatty liver disease: A feature of the metabolic syndrome. *Diabetes* 50: 1844–1850, 2001.

116. Marchesini G, Moscatiello S, Di Domizio S, and Forlani G. Obesity-associated liver disease. *J Clin Endocrinol Metab* 93: S74–80, 2008.

117. Marra F and Bertolani C. Adipokines in liver diseases. *Hepatology* 50: 957–969, 2009.

118. Marra F, DeFranco R, Grappone C, Parola M, Milani S, Leonarduzzi G, Pastacaldi S, Wenzel UO, Pinzani M,

Dianzani MU, Laffi G, and Gentilini P. Expression of monocyte chemotactic protein-1 precedes monocyte recruitment in a rat model of acute liver injury, and is modulated by vitamin E. *J Investig Med* 47: 66–75, 1999.

119. Marra F, Romanelli RG, Giannini C, Failli P, Pastacaldi S, Arrighi MC, Pinzani M, Laffi G, Montalto P, and Gentilini P. Monocyte chemotactic protein-1 as a chemoattractant for human hepatic stellate cells. *Hepatology* 29: 140–148, 1999.

120. Masaki T, Chiba S, Tatsukawa H, Yasuda T, Noguchi H, Seike M, and Yoshimatsu H. Adiponectin protects LPS-induced liver injury through modulation of TNF-alpha in KK-Ay obese mice. *Hepatology* 40: 177–184, 2004.

121. Massip–Salcedo M, Zaouali MA, Padrissa–Altes S, Casillas–Ramirez A, Rodes J, Rosello–Catafau J, and Peralta C. Activation of peroxisome proliferator-activated receptor-alpha inhibits the injurious effects of adiponectin in rat steatotic liver undergoing ischemia-reperfusion. *Hepatology* 47: 461–472, 2008.

122. Matsumoto M, Pocai A, Rossetti L, Depinho RA, and Accili D. Impaired regulation of hepatic glucose production in mice lacking the forkhead transcription factor Foxo1 in liver. *Cell Metab* 6: 208–216, 2007.

123. Mauvais–Jarvis F, Ueki K, Fruman DA, Hirshman MF, Sakamoto K, Goodyear LJ, Iannacone M, Accili D, Cantley LC, and Kahn CR. Reduced expression of the murine p85alpha subunit of phosphoinositide 3-kinase improves insulin signaling and ameliorates diabetes. *J Clin Invest* 109: 141–149, 2002.

124. Michael MD, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA, and Kahn CR. Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. *Mol Cell* 6: 87–97, 2000.

125. Milagro FI, Campion J, and Martinez JA. Weight gain induced by high-fat feeding involves increased liver oxidative stress. *Obesity* 14: 1118–1123, 2006.

126. Moschen AR, Gerner RR, and Tilg H. Pre-B cell colony enhancing factor/NAMPT/visfatin in inflammation and obesity-related disorders. *Curr Pharm Design* 16: 1913–1920, 2010.

127. Muse ED, Lam TK, Scherer PE, and Rossetti L. Hypothalamic resistin induces hepatic insulin resistance. *J Clin Invest* 117: 1670–1678, 2007.

128. Muse ED, Obici S, Bhanot S, Monia BP, McKay RA, Rajala MW, Scherer PE, and Rossetti L. Role of resistin in diet-induced hepatic insulin resistance. *J Clin Invest* 114: 232–239, 2004.

129. Myers MG, Cowley MA, and Munzberg H. Mechanisms of leptin action and leptin resistance. *Annu Rev Physiol* 70: 537–556, 2008.

130. Nakae J, Biggs WH, 3rd, Kitamura T, Cavenee WK, Wright CV, Arden KC, and Accili D. Regulation of insulin action and pancreatic beta-cell function by mutated alleles of the gene encoding forkhead transcription factor Foxo1. *Nat Genet* 32: 245–253, 2002.

131. Nakae J, Cao Y, Daitoku H, Fukamizu A, Ogawa W, Yano Y, and Hayashi Y. The LXXLL motif of murine forkhead transcription factor FoxO1 mediates Sirt1-dependent transcriptional activity. *J Clin Invest* 116: 2473–2483, 2006.

132. Neumeier M, Weigert J, Schaffler A, Weiss TS, Schmidl C, Buttner R, Bollheimer C, Aslanidis C, Scholmerich J, and Buechler C. Aldehyde oxidase 1 is highly abundant in hepatic steatosis and is downregulated by adiponectin and fenofibric acid in hepatocytes in vitro. *Biochem Biophys Res Commun* 350: 731–735, 2006.

133. Novo E and Parola M. Redox mechanisms in hepatic chronic wound healing and fibrogenesis. *Fibrogenesis Tissue Repair* 1: 5, 2008.

134. Osei–Hyiaman D, Liu J, Zhou L, Godlewski G, Harvey–White J, Jeong WI, Batkai S, Marsicano G, Lutz B, Buettner C, and Kunos G. Hepatic CB1 receptor is required for development of diet-induced steatosis, dyslipidemia, and insulin and leptin resistance in mice. *J Clin Invest* 118: 3160–3169, 2008.

135. Parekh S and Anania FA. Abnormal lipid and glucose metabolism in obesity: Implications for nonalcoholic fatty liver disease. *Gastroenterology* 132: 2191–2207, 2007.

136. Pessayre D. Role of mitochondria in non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 22 Suppl 1: S20–27, 2007.

137. Rabe K, Lehrke M, Parhofer KG, and Broedl UC. Adipokines and insulin resistance. *Mol Med* 14: 741–751, 2008.

138. Rahmouni K and Haynes WG. Endothelial effects of leptin: Implications in health and diseases. *Curr Diabetes Rep* 5: 260–266, 2005.

139. Ratziu V, Giral P, Charlotte F, Bruckert E, Thibault V, Theodorou I, Khalil L, Turpin G, Opolon P, and Poynard T. Liver fibrosis in overweight patients. *Gastroenterology* 118: 1117–1123, 2000.

140. Roglans N, Vila L, Farre M, Alegret M, Sanchez RM, Vazquez–Carrera M, and Laguna JC. Impairment of hepatic Stat-3 activation and reduction of PPARalpha activity in fructose-fed rats. *Hepatology* 45: 778–788, 2007.

141. Saito S, Fujiwara T, Matsunaga T, Minagawa K, Fukui K, Fukuda I, Osanai T, and Okumura K. Increased adiponectin synthesis in the visceral adipose tissue in men with coronary artery disease treated with pravastatin: A role of the attenuation of oxidative stress. *Atherosclerosis* 199: 378–383, 2008.

142. Samad F, Pandey M, Bell PA, and Loskutoff DJ. Insulin continues to induce plasminogen activator inhibitor 1 gene expression in insulin-resistant mice and adipocytes. *Mol Med* 6: 680–692, 2000.

143. Samad F, Uysal KT, Wiesbrock SM, Pandey M, Hotamisligil GS, and Loskutoff DJ. Tumor necrosis factor alpha is a key component in the obesity-linked elevation of plasminogen activator inhibitor 1. *Proc Natl Acad Sci USA* 96: 6902–6907, 1999.

144. Samuel VT, Liu ZX, Qu X, Elder BD, Bilz S, Befroy D, Romanelli AJ, and Shulman GI. Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. *J Biol Chem* 279: 32345–32353, 2004.

145. Satapathy SK, Garg S, Chauhan R, Sakhuja P, Malhotra V, Sharma BC, and Sarin SK. Beneficial effects of tumor necrosis factor-alpha inhibition by pentoxifylline on clinical, biochemical, and metabolic parameters of patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 99: 1946–1952, 2004.

146. Satoh H, Nguyen MT, Miles PD, Imamura T, Usui I, and Olefsky JM. Adenovirus-mediated chronic "hyper-resistinemia" leads to *in vivo* insulin resistance in normal rats. *J Clin Invest* 114: 224–231, 2004.

147. Savage DB, Petersen KF, and Shulman GI. Disordered lipid metabolism and the pathogenesis of insulin resistance. *Physiol Rev* 87: 507–520, 2007.

148. Saxena NK, Fu PP, Nagalingam A, Wang J, Handy J, Cohen C, Tighiouart M, Sharma D, and Anania FA. Adiponectin modulates C-Jun N-terminal kinase and mammalian target of rapamycin and inhibits hepatocellular carcinoma. *Gastroenterology* 139: 1762–1773, 2010.

149. Saxena NK, Ikeda K, Rockey DC, Friedman SL, and Anania FA. Leptin in hepatic fibrosis: Evidence for increased collagen production in stellate cells and lean littermates of ob/ob mice. *Hepatology* 35: 762–771., 2002.

150. Saxena NK, Sharma D, Ding X, Lin S, Marra F, Merlin D, and Anania FA. 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* 67: 2497–2507, 2007.

151. Saxena NK, Titus MA, Ding X, Floyd J, Srinivasan S, Sitaraman SV, and Anania FA. Leptin as a novel profibrogenic cytokine in hepatic stellate cells: mitogenesis and inhibition of apoptosis mediated by extracellular regulated kinase (Erk) and Akt phosphorylation. *FASEB J* 18: 1612-1614, 2004.

152. Schattenberg JM, Singh R, Wang Y, Lefkowitch JH, Rigoli RM, Scherer PE, and Czaja MJ. JNK1 but not JNK2 promotes the development of steatohepatitis in mice. *Hepatology* 43: 163–172, 2006.

153. Schenk S, Saberi M, and Olefsky JM. Insulin sensitivity: Modulation by nutrients and inflammation. *J Clin Invest* 118: 2992–3002, 2008.

154. Senn JJ, Klover PJ, Nowak IA, Zimmers TA, Koniaris LG, Furlanetto RW, and Mooney RA. Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. *J Biol Chem* 278: 13740–13746, 2003.

155. Sennello JA, Fayad R, Morris AM, Eckel RH, Asilmaz E, Montez J, Friedman JM, Dinarello CA, and Fantuzzi G. Regulation of T cell-mediated hepatic inflammation by adiponectin and leptin. *Endocrinology* 146: 2157–2164, 2005.

156. Seppala–Lindroos A, Vehkavaara S, Hakkinen AM, Goto T, Westerbacka J, Sovijarvi A, Halavaara J, and Yki–Jarvinen H. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J Clin Endocrinol Metab* 87: 3023–3028, 2002.

157. She H, Xiong S, Hazra S, and Tsukamoto H. Adipogenic transcriptional regulation of hepatic stellate cells. *J Biol Chem* 280: 4959–4967, 2005.

158. Shen Z, Liang X, Rogers CQ, Rideout D, and You M. Involvement of adiponectin-SIRT1-AMPK signaling in the protective action of rosiglitazone against alcoholic fatty liver in mice. *Am J Physiol -Gastroint Liver Physiol* 298: G364–374, 2010.

159. Sierra–Honigmann MR, Nath AK, Murakami C, Garcia–Cardena G, Papapetropoulos A, Sessa WC, Madge LA, Schechner JS, Schwabb MB, Polverini PJ, and Flores–Riveros JR. Biological action of leptin as an angiogenic factor. *Science* (*New York, NY*) 281: 1683–1686, 1998.

160. Singhal NS, Patel RT, Qi Y, Lee YS, and Ahima RS. Loss of resistin ameliorates hyperlipidemia and hepatic steatosis in leptin-deficient mice. *Am J Physiol Endocrinol Metab* 295: E331–338, 2008.

161. Smith SR, Bai F, Charbonneau C, Janderova L, and Argyropoulos G. A promoter genotype and oxidative stress potentially link resistin to human insulin resistance. *Diabetes* 52: 1611–1618, 2003.

162. Soardo G, Donnini D, Varutti R, Moretti M, Milocco C, Basan L, Esposito W, Casaccio D, Stel G, Catena C, Curcio F, and Sechi LA. Alcohol-induced endothelial changes are associated with oxidative stress and are rapidly reversed after withdrawal. *Alcohol Clin Exp Res* 29: 1889–1898, 2005.

163. Song Z, Zhou Z, Deaciuc I, Chen T, and McClain CJ. Inhibition of adiponectin production by homocysteine: A potential mechanism for alcoholic liver disease. *Hepatology* 47: 867–879, 2008.

164. Stefanovic A, Kotur–Stevuljevic J, Spasic S, Bogavac–Stanojevic N, and Bujisic N. The influence of obesity on the oxidative stress status and the concentration of leptin in type 2 diabetes mellitus patients. *Diabetes Res Clin Pract* 79: 156–163, 2008.

165. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, and Lazar MA. The hormone resistin links obesity to diabetes. *Nature* 409: 307–312, 2001.

166. Steppan CM, Brown EJ, Wright CM, Bhat S, Banerjee RR, Dai CY, Enders GH, Silberg DG, Wen X, Wu GD, and Lazar MA. A family of tissue-specific resistin-like molecules. *Proc Natl Acad Sci USA* 98: 502–506, 2001.

167. Suganami T, Nishida J, and Ogawa Y. A paracrine loop between adipocytes and macrophages aggravates inflammatory changes: Role of free fatty acids and tumor necrosis factor alpha. *Arterioscler Thromb Vasc Biol* 25: 2062–2068, 2005.

168. Suganami T, Tanimoto–Koyama K, Nishida J, Itoh M, Yuan X, Mizuarai S, Kotani H, Yamaoka S, Miyake K, Aoe S, Kamei Y, and Ogawa Y. Role of the Toll-like receptor 4/NF-kappaB pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages. *Arterioscler Thromb Vasc Biol* 27: 84–91, 2007.

169. Sugimoto M, Arai H, Tamura Y, Murayama T, Khaengkhan P, Nishio T, Ono K, Ariyasu H, Akamizu T, Ueda Y, Kita T, Harada S, Kamei K, and Yokode M. Mulberry leaf ameliorates the expression profile of adipocytokines by inhibiting oxidative stress in white adipose tissue in db/db mice. *Atherosclerosis* 204: 388–394, 2009.

170. Sun J, Xu Y, Deng H, Sun S, Dai Z, and Sun Y. Intermittent high glucose exacerbates the aberrant production of adiponectin and resistin through mitochondrial superoxide overproduction in adipocytes. *J Mol Endocrinol* 44: 179–185, 2010.

171. Sun X, Han R, Wang Z, and Chen Y. Regulation of adiponectin receptors in hepatocytes by the peroxisome proliferator-activated receptor-gamma agonist rosiglitazone. *Diabetologia* 49: 1303–1310, 2006.

172. Tamura Y, Sugimoto M, Murayama T, Minami M, Nishikaze Y, Ariyasu H, Akamizu T, Kita T, Yokode M, and Arai H. C-C chemokine receptor 2 inhibitor improves diet-induced development of insulin resistance and hepatic steatosis in mice. *J Atheroscler Thromb* 17: 219–228, 2010.

173. Tang M, Potter JJ, and Mezey E. Leptin enhances the effect of transforming growth factor beta in increasing type I collagen formation. *Biochem Biophys Res Commun* 297: 906–911, 2002.

174. Tang Y, Zheng S, and Chen A. Curcumin eliminates leptin's effects on hepatic stellate cell activation via interrupting leptin signaling. *Endocrinology* 150: 3011–3020, 2009.

175. Taniguchi CM, Emanuelli B, and Kahn CR. Critical nodes in signalling pathways: Insights into insulin action. *Nat Rev Mol Cell Biol* 7: 85–96, 2006.

176. Tao L, Wang Y, Gao E, Zhang H, Yuan Y, Lau WB, Chan L, Koch WJ, and Ma XL. Adiponectin: An indispensable molecule in rosiglitazone cardioprotection following myocardial infarction. *Circ Res* 106: 409–417, 2010.

177. Temkin V and Karin M. From death receptor to reactive oxygen species and c-Jun N-terminal protein kinase: The receptor-interacting protein 1 odyssey. *Immunol Rev* 220: 8–21, 2007.

178. Tilg H and Hotamisligil GS. Nonalcoholic fatty liver disease: Cytokine–adipokine interplay and regulation of insulin resistance. *Gastroenterology* 131: 934–945., 2006.

179. Tilg H and Moschen AR. Adipocytokines: Mediators linking adipose tissue, inflammation and immunity. *Nat Rev Immunol* 6: 772–783, 2006.

180. Tokushige K, Takakura M, Tsuchiya–Matsushita N, Taniai M, Hashimoto E, and Shiratori K. Influence of TNF gene polymorphisms in Japanese patients with NASH and simple steatosis. *J Hepatol* 46: 1104–1110, 2007.

181. Tomita K, Azuma T, Kitamura N, Tamiya G, Ando S, Nagata H, Kato S, Inokuchi S, Nishimura T, Ishii H, and Hibi T. Leptin deficiency enhances sensitivity of rats to alcoholic steatohepatitis through suppression of metallothionein. *Am J Physiol Gastroint Liver Physiol* 287: G1078–1085, 2004.

182. Tomita K, Oike Y, Teratani T, Taguchi T, Noguchi M, Suzuki T, Mizutani A, Yokoyama H, Irie R, Sumimoto H, Takayanagi A, Miyashita K, Akao M, Tabata M, Tamiya G, Ohkura T, and Hibi T. Hepatic AdipoR2 signaling plays a protective role against progression of nonalcoholic steatohepatitis in mice. *Hepatology* 48: 458–473, 2008.

183. Tomita K, Tamiya G, Ando S, Ohsumi K, Chiyo T, Mizutani A, Kitamura N, Toda K, Kaneko T, Horie Y, Han JY, Kato S, Shimoda M, Oike Y, Tomizawa M, Makino S, Ohkura T, Saito H, Kumagai N, Nagata H, Ishii H, and Hibi T. Tumour necrosis factor alpha signalling through activation of Kupffer cells plays an essential role in liver fibrosis of non-alcoholic steatohepatitis in mice. *Gut* 55: 415–424, 2006.

184. Tripathy D, Eriksson KF, Orho–Melander M, Fredriksson J, Ahlqvist G, and Groop L. Parallel manifestation of insulin resistance and beta cell decompensation is compatible with a common defect in Type 2 diabetes. *Diabetologia* 47: 782–793, 2004.

185. Tuncman G, Hirosumi J, Solinas G, Chang L, Karin M, and Hotamisligil GS. Functional *in vivo* interactions between JNK1 and JNK2 isoforms in obesity and insulin resistance. *Proc Natl Acad Sci USA* 103: 10741–10746, 2006.

186. Uchida Y, Ohba K, Yoshioka T, Irie K, Muraki T, and Maru Y. Cellular carbonyl stress enhances the expression of plasminogen activator inhibitor-1 in rat white adipocytes via reactive oxygen species-dependent pathway. *J Biol Chem* 279: 4075–4083, 2004.

187. Ueki K, Fruman DA, Yballe CM, Fasshauer M, Klein J, Asano T, Cantley LC, and Kahn CR. Positive and negative roles of p85 alpha and p85 beta regulatory subunits of phosphoinositide 3-kinase in insulin signaling. *J Biol Chem* 278: 48453–48466, 2003.

188. Ueki K, Kondo T, Tseng YH, and Kahn CR. Central role of suppressors of cytokine signaling proteins in hepatic steatosis, insulin resistance, and the metabolic syndrome in the mouse. *Proc Natl Acad Sci USA* 101: 10422–10427, 2004.

189. Valenti L, Rametta R, Dongiovanni P, Maggioni M, Fracanzani AL, Zappa M, Lattuada E, Roviaro G, and Fargion S. Increased expression and activity of the transcription factor FOXO1 in nonalcoholic steatohepatitis. *Diabetes* 57: 1355–1362, 2008.

190. Valfre di Bonzo L, Novo E, Cannito S, Busletta C, Paternostro C, Povero D, and Parola M. Angiogenesis and liver fibrogenesis. *Histol Histopathol* 24: 1323–1341, 2009.

191. Vila L, Roglans N, Alegret M, Sanchez RM, Vazquez–Carrera M, and Laguna JC. Suppressor of cytokine signaling-3 (SOCS-3) and a deficit of serine/threonine (Ser/Thr) phosphoproteins involved in leptin transduction mediate the effect of fructose on rat liver lipid metabolism. *Hepatology* 48: 1506–1516, 2008.

192. Wang Y, Gao E, Tao L, Lau WB, Yuan Y, Goldstein BJ, Lopez BL, Christopher TA, Tian R, Koch W, and Ma XL. AMP-activated protein kinase deficiency enhances myocardial ischemia/reperfusion injury but has minimal effect on the antioxidant/antinitrative protection of adiponectin. *Circulation* 119: 835–844, 2009.

193. Wedemeyer I, Bechmann LP, Odenthal M, Jochum C, Marquitan G, Drebber U, Gerken G, Gieseler RK, Dienes HP, and Canbay A. Adiponectin inhibits steatotic CD95/Fas up-regulation by hepatocytes: Therapeutic implications for hepatitis C. *J Hepatol* 50: 140–149, 2009.

194. Weigert J, Neumeier M, Bauer S, Mages W, Schnitzbauer AA, Obed A, Groschl B, Hartmann A, Schaffler A, Aslanidis C, Scholmerich J, and Buechler C. Small-interference RNA-mediated knock-down of aldehyde oxidase 1 in 3T3-L1 cells impairs adipogenesis and adiponectin release. *FEBS Lett* 582: 2965–2972, 2008.

195. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, and Ferrante AW, Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 112: 1796–1808, 2003.

196. Weston CR and Davis RJ. The JNK signal transduction pathway. *Curr Opin Cell Biol* 19: 142–149, 2007.

197. Wojcicka G, Jamroz–Wisniewska A, Widomska S, Ksiazek M, and Beltowski J. Role of extracellular signal-regulated kinases (ERK) in leptin-induced hypertension. *Life Sci* 82: 402–412, 2008.

198. Wu B, Fukuo K, Suzuki K, Yoshino G, and Kazumi T. Relationships of systemic oxidative stress to body fat distribution, adipokines and inflammatory markers in healthy middle-aged women. *Endocr J* 56: 773–782, 2009.

199. Xu A, Wang Y, Keshaw H, Xu LY, Lam KS, and Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *J Clin Invest* 112: 91–100, 2003.

200. Xu Y, Rojkind M, and Czaja MJ. Regulation of monocyte chemoattractant protein 1 by cytokines and oxygen free radicals in rat hepatic fat-storing cells. *Gastroenterology* 110: 1870–1877, 1996.

201. Yamaguchi K, Yang L, McCall S, Huang J, Yu XX, Pandey SK, Bhanot S, Monia BP, Li YX, and Diehl AM. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. *Hepatology* 45: 1366–1374, 2007.

202. Yamauchi T, Nio Y, Maki T, Kobayashi M, Takazawa T, Iwabu M, Okada-Iwabu M, Kawamoto S, Kubota N, Kubota T, Ito Y, Kamon J, Tsuchida A, Kumagai K, Kozono H, Hada Y, Ogata H, Tokuyama K, Tsunoda M, Ide T, Murakami K, Awazawa M, Takamoto I, Froguel P, Hara K, Tobe K, Nagai R, Ueki K, and Kadowaki T. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. *Nat Med* 13: 332–339, 2007.

203. Yanagawa Y, Morimura T, Tsunekawa K, Seki K, Ogiwara T, Kotajima N, Machida T, Matsumoto S, Adachi T, and Murakami M. Oxidative stress associated with rapid

weight reduction decreases circulating adiponectin concentrations. *Endocr J* 57: 339–345, 2010.

204. Yang SJ, IglayReger HB, Kadouh HC, and Bodary PF. Inhibition of the chemokine (C-C motif) ligand 2/chemokine (C-C motif) receptor 2 pathway attenuates hyperglycaemia and inflammation in a mouse model of hepatic steatosis and lipoatrophy. *Diabetologia* 52: 972–981, 2009.

205. Yang SQ, Lin HZ, Lane MD, Clemens M, and Diehl AM. Obesity increases sensitivity to endotoxin liver injury: Implications for the pathogenesis of steatohepatitis. *Proc Natl Acad Sci USA* 94: 2557–2562, 1997.

206. Yi W, Sun Y, Gao E, Wei X, Lau WB, Zheng Q, Wang Y, Yuan Y, Wang X, Tao L, Li R, Koch W, and Ma XL. Reduced cardioprotective action of adiponectin in high-fat diet–induced type II diabetic mice and its underlying mechanisms. *Antioxid Redox Signal* 2011. [Epub ahead of print]; DOI: 10.1089/ars.2010.3722.

207. You M, Considine RV, Leone TC, Kelly DP, and Crabb DW. Role of adiponectin in the protective action of dietary saturated fat against alcoholic fatty liver in mice. *Hepatology* 42: 568–577, 2005.

208. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, and Shoelson SE. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* 293: 1673–1677, 2001.

209. Zamara E, Galastri S, Aleffi S, Petrai I, Aragno M, Mastrocola R, Novo E, Bertolani C, Milani S, Vizzutti F, Vercelli A, Pinzani M, Laffi G, LaVilla G, Parola M, and Marra F. Prevention of severe toxic liver injury and oxidative stress in MCP-1-deficient mice. *J Hepatol* 46: 230–238, 2007.

210. Zhan SS, Jiang JX, Wu J, Halsted C, Friedman SL, Zern MA, and Torok NJ. Phagocytosis of apoptotic bodies by hepatic stellate cells induces NADPH oxidase and is associated with liver fibrosis *in vivo*. *Hepatology* 43: 435–443, 2006.

211. Zhang LP, Takahara T, Yata Y, Furui K, Jin B, Kawada N, and Watanabe A. Increased expression of plasminogen activator and plasminogen activator inhibitor during liver fibrogenesis of rats: Role of stellate cells. *J Hepatol* 31: 703–711, 1999.

212. Zhou H, Song X, Briggs M, Violand B, Salsgiver W, Gulve EA, and Luo Y. Adiponectin represses gluconeogenesis independent of insulin in hepatocytes. *Biochem Biophys Res Commun* 338: 793–799, 2005.

213. Zhou Y, Jia X, Wang G, Wang X, and Liu J. PI-3 K/AKT and ERK signaling pathways mediate leptin-induced inhibition of PPARgamma gene expression in primary rat hepatic stellate cells. *Mol Cell Biochem* 325: 131–139, 2009.

Address correspondence to:
Prof. Fabio Marra
Dipartimento di Medicina Interna
Università degli Studi di Firenze
Viale Morgagni 85
50134, Firenze
Italy

E-mail: f.marra@dmi.unifi.it

Abbreviations Used

5-LOX = 5-lipoxygenase
AMPK = AMP-activated protein kinase
ChREBP = carbohydrate response element-binding protein
eNOS = endothelial NO synthase
FFA = free fatty acids
FOXO1 = forkhead box–containing protein O subfamily-1
GSK3 = glycogen-synthase kinase 3
HCC = hepatocellular carcinoma
HSC = hepatic stellate cells
IKK = IκB kinase
IR = insulin receptor
IRS = insulin receptor substrate
JNK = c-jun N-terminal kinase
LPS = lipopolysaccharide
MAPK = mitogen-activated protein kinase
MCP-1 = monocyte chemoattractant protein-1
mTOR = mammalian target of rapamycin
NAFLD = nonalcoholic fatty liver disease
NASH = nonalcoholic steatohepatitis
PEPCK = phosphoenolpyruvate carboxykinase
PI3K = phosphatidylinositol 3-kinase
PPAR = peroxisome proliferator-activated receptor
ROS = reactive oxygen species
RTK = receptor tyrosine kinase
SOCS = suppressor of cytokine signaling
SREBP-1 = sterol regulatory element binding protein-1
STAT = signal transducer and activator of transcription
TBP-2 = thioredoxin-binding protein-2
TG = triglycerides
TSC = tuberous sclerosis complex