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Fatty liver and chronic kidney disease: novel mechanistic insights and therapeutic opportunities

Running title: NAFLD and CKD

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Abstract

Chronic kidney disease (CKD) is a risk factor for end-stage renal disease (ESRD) and cardiovascular disease (CVD). A substantial proportion of CKD patients receiving standard-of-care therapy develops ESRD or CVD and mortality in CKD remains unchanged. These data suggest key pathogenetic mechanisms underlying CKD progression go unaffected by current treatments. Growing evidence suggests non-alcoholic fatty liver disease (NAFLD) and CKD share common pathogenetic mechanisms and potential therapeutic targets, which will be discussed. Common nutritional conditions predisposing to both NAFLD and CKD include excessive fructose intake and vitamin D deficiency. Modulation of nuclear transcription factors regulating key pathways of lipid metabolism, inflammation and fibrosis, including Peroxisome Proliferator-activated Receptors (PPARs) and Farnesoid X Receptor (FXR) is advancing to stage III clinical development. The relevance of epigenetic regulation in the pathogenesis of NAFLD and CKD is also emerging, and modulation of miRNA21 is a promising therapeutic target.

While single antioxidant supplementation yielded variable results, modulation of key effectors of redox regulation and of molecular sensors of intracellular energy, nutrient or oxygen status gave promising preclinical results.

Other emerging therapeutic approaches target key mediators of inflammation, like chemokines, and of fibrogenesis, like galectin-3, or gut dysfunction through gut microbiota manipulation and incretin based therapies. Furthermore, NAFLD *per se* affects CKD through lipoprotein metabolism and hepatokine secretion and, conversely, targeting the renal tubule by Sodium-Glucose Linked Transporter-2 inhibitors can improve both CKD and NAFLD.

Implications for the treatment of NAFLD and CKD are discussed in light of this new therapeutic armamentarium.

Epidemiological evidence linking NAFLD and CKD

Chronic kidney disease (CKD) affects up to 8% of the world's adult population, with its prevalence increasing in a population that is ageing and beset by lifestyle-associated diseases such as obesity, metabolic syndrome, diabetes, hypertension, and smoking (1).

CKD may progress to end-stage renal disease (ESRD) and is an important cardiovascular disease (CVD) risk factor: importantly, most patients with CKD die from CVD before renal replacement therapy is initiated (1).

There is potential for improving recognition and treatment of CKD: in the Third National Health and Nutrition Survey (NHANES III), awareness among stage 3 CKD patients was lower than 8% (1).

Furthermore, a substantial proportion of CKD patients receiving standard-of-care therapy develop ESRD or CVD and all-cause mortality remains unchanged in CKD population (2). These data suggest key pathogenetic mechanisms underlying renal disease progression go unaffected by current treatment and prompt search for easily identifiable risk factors and novel pharmacological targets.

The presence and severity of non-alcoholic fatty liver disease (NAFLD) has been recently related to the incidence and stage of CKD (3), independently of traditional CKD risk factors; conversely, the presence of CKD increased overall mortality in patients with NAFLD as compared to the general population (4). Further supporting a pathogenic link between NAFLD and CKD, NASH-related cirrhosis has a higher risk of renal failure than other aetiologies of cirrhosis, is an increasing indication for simultaneous liver-kidney transplantation and an independent risk factor for kidney graft loss and CVD (5,6).

Collectively, these data suggest common pathogenic mechanisms subtend both liver and kidney injury and could be targeted to retard the progression of both NAFLD and CKD.

Potential pathogenic mechanisms contributing to NAFLD to CKD and therapeutic implications

Nutritional factors in NAFLD and CKD: fructose and vitamin D intake

Dietary intake of fructose, the main constituent of sugar-sweeteners, increased 2-fold over the last decade (7). Fructose may contribute to liver and kidney injury through several mechanisms, including uric acid overproduction and consistently, uric acid lowering agents improved fructose-induced experimental NAFLD and CKD (**Table 1**) (8,9). On this basis, the impact of xanthine oxidase inhibitors on CKD progression is being evaluated in the trials CKD-FIX (clinicaltrials.gov ID: NCT12611000791932) and FEATHER (FEbuxostat versus placebo rAndomized controlled Trial regarding reduced renal function in patients with Hyperuricemia complicated by chRonic kidney disease stage 3, UMIN ID: UMIN000008343) (**Table 2**).

Vitamin D attracted considerable interest because of its “pleiotropic” functions, with roles in regulation of cell proliferation, differentiation, immunity, inflammation, fibrogenesis and metabolism (**Table 1**), concurrent with an unsuspectedly high prevalence of vitamin D deficiency, approaching 25% of the general adult population (10). Vitamin D deficiency has been linked to the pathogenesis and severity of NAFLD and CKD by observational and experimental data (**Table 1**) (11-13). However, the few trials with vitamin D supplementation yielded mixed results and the benefit of vitamin D supplementation remains uncertain (14). Concerning NAFLD and CKD, it should be noted that these conditions are characterized by vitamin D resistance, partly determined by impaired hepatic 25 hydroxylation and increased renal tubular 25(OH)D loss, and may require higher dose supplementation of vitamin D, calcitriol or vitamin D receptor agonists (e.g. paricalcitol) to be overcome (15, 16). The effects of vitamin D supplementation in CKD and in NASH are being evaluated in RCTs NCT00893451, NCT01623024 and NCT02098317.

Reversing ectopic fat deposition by targeting nuclear transcription factors in NAFLD and CKD

NAFLD and CKD are characterized by ectopic toxic lipid accumulation, which is determined by an extensive derangement in hepatic and renal lipid metabolism and triggers lipoperoxidative stress, cell apoptosis, inflammation and fibrosis (17,18,19) (**Figure 1**). These abnormalities are subtended by an

extensive deregulation of nuclear transcription factors regulating lipid metabolism, inflammation and fibrogenesis, including Peroxisome Proliferator-Activated Receptor (PPAR)- α , PPAR- δ and PPAR- γ , Sterol Regulatory Binding Protein (SREBP)-2 and Farnesoid X Receptor (FXR), which represent an attractive target for the treatment of NAFLD and CKD(20,21)(**Figure 1**).

Based on the finding that PPAR- α and PPAR- δ are down-regulated in NAFLD and CKD(20,22), potent, selective PPAR- α , PPAR- δ and dual PPAR- α/δ agonists (K-877, GW501516, MBX-8025 and GFT505, respectively) were evaluated in these two conditions, with encouraging results in preclinical models (22,23,24). Some of these compounds advanced to the clinical stage of development, and GFT505, a dual PPAR α/δ agonist, improved steatohepatitis, fibrosis and glyco-lipid profile in the recently completed GOLDEN-505 trial (25)(**Table 2**).

The PPAR- γ agonists thiazolidinediones are another pharmacological class that significantly improved NASH and albuminuria in clinical trials (26, 27), but whose clinical use was limited by their side effects. These drawbacks prompted development of new compounds, including dual PPAR- α/γ agonists, which maintained the therapeutic effectiveness of PPAR- γ agonists but were devoid of their unwanted effects (**Figure 1**). Saroglitazar, a potent PPAR- α/γ agonist, did not induce weight gain, peripheral edema or other adverse events after 1 year (28) and improved markers of NAFLD in diabetic patients (29), while aleglitazar slowed eGFR decline in diabetic nephropathy (30)(**Table 2**). A small, phase IIa trial enrolling biopsy-proven NASH patients has been completed but results are not available yet (CTRI registration no.: CTRI/2010/091/000108).

Larger and longer RCTs are needed to evaluate long-term clinical safety and effectiveness of these compounds in diabetic and non-diabetic patients.

Among different lipotoxic species accumulating in NAFLD and CKD, free cholesterol is believed to play a key pathogenic role in liver and renal injury (31,32). Ectopic cholesterol accumulation is driven by an inappropriate upregulation of transcription factor SREBP-2, with consequently increased cholesterol synthesis, influx and retention and reduced cholesterol excretion by liver and renal cells(31,32)(**Figure 1**). Such pervasive deregulation in all steps of cholesterol metabolism may diminish the effectiveness of available cholesterol-lowering drugs, that target single steps in

cholesterol metabolism (33). Therefore, modulation of SREBP-2 activity represents an attractive therapeutic tool: while selective SREBP-2 antagonists are under development, several natural antioxidants, like myricetin, repressed SREBP-2 expression and ameliorated cholesterol-induced inflammation and fibrosis in experimental models(32)(**Table 2**).

Farnesoid X Receptor (FXR) is a nuclear transcription factor with prominent insulin sensitizing, anti-lipogenic, anti-inflammatory and antifibrotic properties; furthermore, FXR activation improves also endothelial function (34,35,36)(**Figure 1**).

FXR expression is down-regulated in the liver and kidney of NAFLD and CKD patients, respectively, and inversely related to disease severity. On this basis, potent semi-synthetic bile acid FXR agonists have been developed (**Table 2**). Obeticholic acid (OCA, or INT-747), a semi-synthetic chenodeoxycholic acid derivative, improved liver histology in the phase IIa multicenter, randomized “FXR Ligand NASH Treatment (FLINT)” trial and ameliorated renal histology and proteinuria in nutritional models of CKD (35,36). Several issues remain, however, including the effectiveness of FXR agonists in nondiabetic subjects and the impact of HDL-C reduction on long-term CVD risk of these patients.

Epigenetic regulation in NASH and CKD

MicroRNAs (miRNAs) are small (~22 base-pairs) endogenous non-coding RNAs

that regulate gene expression of at least 60% of protein-coding genes.

MicroRNA recognize mRNA targets through sequence complementarity between the miRNA and binding sites in the 3' untranslated regions (3' UTRs) of the target mRNAs or through interaction with RNA-binding proteins.

MicroRNAs regulate gene expression in one of two ways, depending on the degree of complementarity between the miRNA and its target: miRNAs that bind to mRNA targets with

imperfect complementarity block target gene expression via translational silencing, while miRNAs binding their mRNA targets with perfect complementarity enhance target gene expression (38, 39). To date, over 2500 miRNAs have been identified in the human genome and each miRNA can regulate several hundred target genes involved in diverse developmental and cellular processes, including cellular metabolism proliferation, differentiation and apoptosis.

Several miRNAs have been found to be dysregulated in NAFLD and CKD (38,39). On this basis, approaches inhibiting overexpressed miRNAs by antisense oligonucleotides (ASOs) or restoring the expression of down-regulated miRNAs by synthetic miRNA mimics have been attempted.

miRNA21, in particular, is an attractive target, as it regulates key metabolic, pro-inflammatory and profibrogenic pathways and its hepatic and renal overexpression in NASH and CKD leads to PPAR- α downregulation, SREBP-2 upregulation, mitochondrial dysfunction and pro-fibrogenic HSC activation and proximal tubular cell epithelial-to-mesenchymal transition(EMT)(40,41).

Consistently, in experimental models of NASH and CKD, anti-microRNA-21 ASOs induced weight loss, normalized metabolic dysregulation, and improved hepatic and renal inflammation and fibrosis, effects at least partly mediated by PPAR- α upregulation (40,41).

Despite these premises, several issues remain, including the stability and selective delivery of the pharmacological modulators to the target organs, and long-term safety of this approach, as miRNAs regulate also cell proliferation and cell cycle progression in diverse tissues and long-term consequences of its modulation on tumor onset and progression are unclear (39,40).

Role of cellular energy, oxygen and nutrient sensors

In mammals, cellular metabolism is finely orchestrated by molecular sensors of energy, nutrient and oxygen status to adapt to changing substrate availability. The dysregulation of some of these sensors, including 5-AMP-activated protein kinase (AMPK), hypoxia-inducible factor (HIF)-1 α and mammalian target of rapamycin (mTOR), has been implicated in the pathogenesis of NAFLD and CKD, and could be targeted for the treatment of NAFLD and CKD.

AMPK is an ubiquitous kinase that preserves cell survival under calorie restriction or high energy demand (42). In response to cellular ATP depletion or to an increase in AMP/ATP ratio, AMPK activation enhances substrate oxidation and has anti-oxidant, anti-inflammatory and antifibrotic effects (43-45) (**Figure 2A**). On this basis, several natural AMPK activators were assessed in preclinical models of NASH and CKD, with encouraging results (43-45), and the synthetic AMPK-activator oltipraz is being evaluated in non-cirrhotic NAFLD patients (clinicaltrials.gov ID: NCT01373554)(**Table 2**).

HIF-1 α is a ubiquitous oxygen-sensitive protein that regulates transcription of genes involved in metabolic adaptation, energy conservation, angiogenesis, and cell survival in response to cellular hypoxia and to non-hypoxic stimuli like cholesterol overload (46). Inappropriate HIF-1 α activation following stimuli like chronic intermittent hypoxia and cholesterol overload has been involved in NASH pathogenesis (47,48), while chronic hypoxia has been implicated in renal injury in early diabetic and obesity-related CKD (49, 50) (**Figure 2B**).

On this basis, small-molecule synthetic HIF-1 α inhibitors, including YC-1, have been developed and showed potent anti-fibrotic properties in experimental models of NASH (51)(**Table 2**).

Several issues remain, however: in some studies HIF-1 α activation protected against kidney injury (52), suggesting HIF-1 α may not be the sole, or the best therapeutic target for reversing cellular effects of hypoxia in CKD.

mTOR is a serine/threonine kinase that, in response to changes in cellular nutrient levels, growth factors like insulin and IGF, and other stressors, associates with companion proteins to form two distinct signaling molecular complexes, mTOR complex 1 (mTORC1) and mTORC2 (53). mTORC1 has been more extensively studied and has been found to promote cellular anabolism by stimulating synthesis of protein, lipid, and nucleotides and blocking catabolic processes. Inappropriate mTORC1 activation in NAFLD and CKD inhibits autophagy (53,54) and promotes insulin resistance, ectopic lipid accumulation, lipotoxicity and proinflammatory monocyte recruitment in the liver and kidney (55,56)(**Figure 2B**). Consistently, mTORC1 inhibition reversed metabolic abnormalities and

attenuated lipid accumulation, inflammation, and fibrosis in diverse models of NASH and CKD (57,58) and may represent a therapeutic option for NAFLD and CKD (59)(**Table 2**).

Targeting redox regulation in the pathogenesis of NAFLD and CKD

Although increased oxidative stress is believed to play a central role in NASH and CKD progression, single antioxidant supplementation strategies yielded variable results (26), and other approaches targeting common effectors of redox regulation, like Apoptosis Signal-Regulating Kinase 1 (ASK1) and Nuclear Erythroid 2-related Factor 2 (Nrf2), are being investigated.

ASK1 is a serine/threonine kinase belonging to the mitogen-activated protein kinase kinase kinase (MAP3Ks) family, which is activated in response to stresses like ROS, TNF- α , lipopolysaccharide (LPS) and ER stress (60). ASK1 activates downstream terminal MAPK kinases p38 and c-Jun N-terminal kinase (JNK), which promote insulin resistance, cell death, proinflammatory cytokine/chemokine production, and fibrogenesis(60)(**Figure 3**).

Recent data implicated ASK1 activation in oxidative stress-induced inflammation and fibrogenesis in NASH and CKD, and pharmacological ASK1 inhibition prevented diet-induced NASH and halted progression of diabetic and nondiabetic experimental CKD(61,62). On this basis, the highly selective oral ASK1 inhibitor GS-4997 is being evaluated in NASH patients with moderate-advanced fibrosis (ClinicalTrials.gov ID: NCT02466516) and in diabetic patients with stage 3/4 nephropathy (ClinicalTrials.gov ID: NCT02177786)(**Table 2**).

Several issues need to be clarified: ASK1 inhibition did not improve podocyte loss and albuminuria in experimental diabetic CKD, suggesting this kinase is not central for glomerular injury (62). Furthermore, the impact of ASK1 inhibitors in nondiabetic CKD is unknown.

Nrf2 is a transcription factor expressed ubiquitously in human tissues and most abundantly in the liver (63), where it regulates the expression of several antioxidant and detoxifying enzymes and has direct metabolic, anti-inflammatory and pro-autophagic actions (63)(**Figure 3**). Under basal conditions Nrf2 is kept transcriptionally inactive through binding to its inhibitor, Kelch-like ECH-associated protein 1

(KEAP1) (63). ROS and reactive nitrogen species (RNS) interact with KEAP1 and cause loosening of Nrf-2, which translocates to the nucleus and modulates transcription of its target genes (**Figure 3**). The relevance of Nrf2 in the pathogenesis of liver and kidney injury has emerged in diverse diet-induced models of NASH and CKD, where disease progression was accelerated by Nrf2 deletion and prevented by Nrf2 activators (64,65). On this basis, several natural and synthetic small-molecule Nrf2 activators are currently being evaluated in NASH and CKD patients (**Table 2**): following early encouraging data, clinical development of bardoxolone methyl, a synthetic Nrf2 activator, was interrupted for safety concerns related to heart failure (66). A reanalysis of the potential risk/benefit ratio of the drug for the Japanese population and the observation that most of the severe adverse effects occurred in the first month of therapy prompted initiation of a dose-escalating RCT in Japan (clinicaltrials.gov ID: NCT02316821). Another potent synthetic Nrf2 activator, oltipraz, is being evaluated in NAFLD patients (clinicaltrials.gov ID: NCT01373554).

Targeting molecular effectors of inflammation and fibrosis

Chemokines are small proteins that regulate leukocyte migration into tissues and consequent inflammation, tissue remodeling and fibrosis (67). Among the over 40 chemokine ligands and 20 chemokine receptors currently identified, chemokine (C-C motif) ligand 2 (CCL2, or monocyte chemoattractant protein-1, MCP-1) and its receptor CCR2 have been implicated in the pathogenesis of NASH and CKD. In NAFLD, hepatic cells and adipocytes secrete CCL2, which attracts pro-inflammatory cells to the liver to promote NASH development (68,69), while genetic or pharmacological inhibition of CCL2/CCR2 axis reverses steatohepatitis and advanced hepatic fibrosis (69). In the kidney, tubule cells and podocytes secrete chemokines CCL2 and CCL5 in response to diverse pro-inflammatory stimuli to promote tubulo-interstitial inflammation and fibrosis, which are all reversed by chemokine antagonists (70). On this basis, chemokine antagonists are advancing to clinical stage of development: the small molecule CCR2 antagonist CCX140-B reduced albuminuria and slowed eGFR decline in diabetic nephropathy (71), while the dual chemokine receptor CCR2/CCR5 antagonists BMS-813160, PF-04634817 and cenicriviroc are being evaluated in

diabetic nephropathy(ClinicalTrials.gov ID: NCT01752985, NCT01712061) and in NASH (ClinicalTrials.gov ID: NCT02217475)(**Table 2**).

Galectin-3 is a lectin broadly expressed by immune and epithelial cells, where it localizes mainly in the cytoplasm, but also in the nucleus, in the cell surface and in the extracellular space (72). Galectin-3 regulates cell proliferation, apoptosis, cell adhesion and affinity for advanced glycation end-products(AGEs), exerting multiple and sometimes contrasting effects depending on its cellular location, cell type and mechanism(s) of injury(72)(**Figure 3**).

Galectin-3 is upregulated in the liver and kidney of patients with NASH and CKD and correlates with the severity of liver and renal disease (73,74). Furthermore, circulating galectin-3 levels predict renal function decline, cardiovascular and all-cause mortality in CKD patients (75). Consistent with epidemiological data, functional galectin-3 manipulation disclosed important pro-inflammatory and profibrotic effects of this lectin (72-74).

On this basis, pharmacological galectin-3 inhibition with small molecule competitive inhibitors, including GR-MD-02(galactoarabino-rhamnogalaturonan), GM-CT-01(galactomannan), and N-acetyllactosamine, prevented hypertensive nephropathy (76) and reversed diet-induced NASH and cirrhosis (77). GR-MD-02 was well tolerated and improved markers of hepatic fibrosis in a phase I RCT enrolling NASH patients with advanced fibrosis(ClinicalTrials.gov ID: NCT01899859), while a phase IIa RCT is exploring the effect of the galectin-3 antagonist GCS-100 on eGFR in CKD (ClinicalTrials.gov ID: NCT01843790) (**Table 2**).

Data on galectin-3 inhibition are not univocal, however, and galectin-3 deletion exacerbated systemic inflammation, hyperglycemia, liver and kidney injury in diet-induced obese rodents (78). It has been suggested that inhibition of AGE uptake by the liver, which clears >90% of these end-products from the circulation, promotes their systemic accumulation and RAGE-mediated uptake by other tissues, thereby aggravating extrahepatic toxicity of these molecules. As both NASH and CKD are characterized by AGE accumulation, a better understanding of the impact of galectin-3 inhibitors on AGE-mediated tissue injury is warranted *in vivo*.

The gut connection: targeting incretins and gut microbiota for the treatment of NAFLD and CKD

Incretin based therapies, including glucagon-like peptide-1 (GLP-1) mimetics and dipeptidyl peptidase-4 (DPP-4) inhibitors, increase insulin release from the pancreas, reduce glucagon production and possess numerous extrapancreatic metabolic benefits which prompted evaluation for the treatment of NAFLD and CKD(79,80)(**Table 3**).

Preliminary human data suggest incretin mimetics may improve NAFLD: a meta-analysis of 6 RCTs from the “Liraglutide Effect and Action in Diabetes” (LEAD) program found a significant improvement in biochemical and radiological features of steatosis (81). Furthermore, in the “Liraglutide Efficacy and Action in NASH” (LEAN) trial, liraglutide 1.8 mg/day for 48 weeks induced NASH resolution and improved markers of lipotoxicity, inflammation and metabolic dysfunction as compared with placebo (82)(**Table 2**).

Incretin-based therapies have also the potential for nephroprotection independent of improved glycemic control, through several mechanisms(**Table 3**). GLP-1 administration induced natriuresis through inhibition of proximal tubular Na-H exchanger 3 (NHE3) and reduced activation of the AngII axis (83). These actions counteract the increase in proximal tubular Na reabsorption, which has been hypothesized to trigger glomerular hyperfiltration, the functional defect thought central to obesity-associated and diabetic CKD (83). Furthermore, GLP-1 mimetics demonstrated direct renal anti-inflammatory, anti-fibrotic and antioxidative actions(84,85). Collectively, these properties may explain the attenuated progression of overt diabetic nephropathy in 2 preliminary small RCTs of treatment with liraglutide (84,85).

In addition to inactivating GLP-1, DPP-4 cleaves multiple other peptides, including brain-derived natriuretic peptide (BNP), neuropeptide Y (NPY), peptide YY (PYY) and stromal-derived factor (SDF)-1a(**Table 3**). Thus, the effect of DPP-4 inhibition depends on the actions of the different substrates inactivated in each tissue and organ. This may theoretically explain the slightly lower anti-hypertensive effect observed with DPP-4 inhibitors as compared to GLP-1 mimetics (86).

While the impact of DPP-4 inhibitors on NAFLD has to be assessed, results from the Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus–Thrombolysis in Myocardial Infarction (SAVOR-TIMI) 53 trial (87) and from other 4 RCTs (88) suggest saxagliptin and linagliptin may reduce the development and progression of albuminuria. Renal effects of linagliptin are currently being investigated in the MARLINA-T2D (efficacy, safety and modifications of albuminuria in T2DM subjects with renal disease with LINAgliptin (clinicaltrials.gov ID: NCT01792518) and in the CARMELINA (Cardiovascular safety and renal microvascular outcome study with LINAgliptin (clinicaltrials.gov ID: NCT01897532)) trials.

In conclusion, incretin-based therapies address several of the pathophysiological mechanisms common to experimental NAFLD and CKD, but their impact on renal disease in non-diabetic CKD is unknown. Furthermore, incretin-based therapies did not affect the risk of CVD, a major cause of death in NAFLD and CKD (89).

The capacity for gut microbiota to interact with host metabolic and immune response and to contribute to the development of obesity-associated disorders is being increasingly recognized (90). Both NAFLD and CKD patients exhibit an altered gut microbiota composition, with a relative decrease in “healthy” Bacteroidetes, Lactobacillaceae and Prevotellaceae families and disruption of the normal gastrointestinal barrier (91,92). The resulting accumulation of gut-derived toxins induces inflammation (93), insulin resistance and ectopic fat deposition in liver and muscle through several mechanisms (**Table 3**). Some of these molecules, including endotoxin, indoxyl-sulphate, p-Cresyl sulphate (p-CS) and trimethylamine-N-oxide (TMAO), have documented clinical relevance for the development and progression of CKD (94-97).

CKD may also *per se* aggravate gut dysbiosis and systemic inflammation through accumulation of uremic toxic metabolites (URMs) normally eliminated by the kidneys, including urea and p-CS. The accumulation of urea may lead to influx into the gastrointestinal lumen, where it is hydrolysed to ammonia by microbial urease, then converted to ammonium hydroxide. Ammonia and ammonium hydroxide promote the growth of urea-metabolizing bacteria at the expense of carbohydrate-fermenting strains and disrupt intestinal epithelial tight junctions, enhancing passage of LPS and other

toxic luminal compounds into the circulation (98). Further highlighting the relevance of this mechanism to systemic inflammation, administration of oral activated charcoal absorbent AST-120 improved intestinal barrier function, and reduced systemic oxidative stress, inflammation and endotoxemia in rodent models of CKD (98). Gut microbiota manipulation with probiotics or prebiotics improved surrogate markers of NAFLD in small RCTs of short duration (99) and reduced URM levels in CKD patients (100). The impact of synbiotic administration on renal function in CKD is being investigated in the SYNbiotics Easing Renal failure by improving Gut microbiology (SYNERGY) trial (Australian New Zealand Clinical Trials Registry Number: ACTRN12613000493741).

NAFLD as a determinant of CKD: targeting the liver to improve CKD

In NAFLD, liver disease *per se* contributes to kidney injury through several mechanisms: the liver contains up to 80% of all macrophages of the body and the steatotic liver may represent a more relevant source of proinflammatory cytokines than adipose tissue (101). Furthermore, the liver is a central regulator of lipoprotein metabolism and secretes hepatokines like FGF21, which can modulate whole body metabolism and inflammation.

1) hepatic secretion of VLDL, CETP and syndecan-1 in the pathogenesis of atherogenic dyslipidemia and kidney injury.

Atherogenic dyslipidemia is the commonest lipid abnormality in CKD and an independent predictor of the incidence and progression of CKD and of CVD in CKD patients (102,103). Atherogenic dyslipidemia promotes CKD through receptor-mediated uptake of qualitatively abnormal lipoproteins by glomerular and tubulo-interstitial cells (104). NAFLD may promote atherogenic dyslipidaemia through several mechanisms, which represent potential therapeutic targets: liver fat accumulation *per se* proportionally increases hepatic secretion rate of large VLDL1, which exchange Tg with cholesterol contained in circulating LDL and HDL particles, resulting in sLDL and HDL3 formation (31). Furthermore, recent studies demonstrated circulating cholesteryl ester transfer protein (CETP)

derives largely from hepatic Kupffer cells and its levels parallel the severity of histological necroinflammations in NASH (105). Intriguingly, CETP inhibitors alleviated high fat diet-induced steatohepatitis and fibrosis, possibly by reducing oxLDL uptake by hepatic Kupffer and stellate cells (106) and represent a potential therapeutic target for the treatment of both NAFLD and CKD(**Table 2**).

Syndecan-1 is another key mediator of hepatic clearance of triglyceride-rich lipoproteins (107): syndecan-1 is a transmembrane heparan sulfate proteoglycan constitutively bound to hepatocyte membrane, where it binds LPL and apoE through its heparan sulfate chains and internalizes apoE-containing lipoproteins (108). Sulfation by hepatic sulfotransferases and binding to hepatocyte membrane are required for syndecan-1 biological activity. NAFLD is characterized by an increased shedding of syndecan-1 (109), as a result of increased hepatic metalloproteinase activity, and by a defective syndecan-1 sulfation, as a result of defective hepatic sulfotransferase activity (110, 111). These changes in hepatic syndecan-1 metabolism impair TRLP clearance and, accordingly, predicted atherogenic dyslipidemia in CKD (112). Beside mediating hepatic TRLP clearance, syndecan-1 is also a key constituent of endothelial glycocalyx layer and its shedding has been associated with loss of endothelial barrier integrity and endothelial dysfunction across progressive CKD stages (113). Inhibitors of syndecan-1 shedding, including the phospholipid sphingosine-1-phosphate, restored endothelial integrity experimentally (114) and may represent a potential therapeutic tool for the treatment of CKD.

2)Fibroblast growth factor(FGF)21

Fibroblast growth factors (FGFs) are a group of signalling proteins that regulate embryonic development, tissue regeneration, and diverse metabolic functions by binding extracellularly to four cell surface tyrosine kinase FGF receptors(FGFRs 1–4) (115). FGF21 is mainly secreted by the liver and exerts its multiple beneficial metabolic effects by binding to FGFRs in the presence of co-receptor β -Klotho(115): FGF21 administration ameliorates adipose and hepatic insulin sensitivity, suppresses hepatic gluconeogenesis and lipogenesis and enhances FFA oxidation and mitochondrial

function, at least in part by activating the AMPK-SIRT1-PGC-1 α pathway (115, 116). Furthermore, FGF-21 has recently demonstrated direct anti-inflammatory and anti-fibrogenic activity by inhibiting the key NF- κ B and TGF- β /smad2/3 signaling pathways (117). By virtue of these properties, FGF21 administration improved experimental NASH and CKD (115-117). The clinical development of FGF21 however, is hampered by its short half-life (0.5-5 hr) and by tissue FGF21 resistance, which is subtended by FGFR1 and β -Klotho downregulation (118) and is overcome by pharmacological doses of FGF21. To this aim, engineering of native molecule yielded FGF21 analogs with improved biophysical properties and one of these FGF21 analogs, LY2405319, ameliorated atherogenic dyslipidemia, insulin resistance and adiponectin in obese diabetic patients (119).

Targeting the /renal tubule to improve CKD and NAFLD

Sodium-Glucose Linked Transporter-2 (SGLT2) inhibitors block the activity of the SGLT2 protein, which is expressed in the S1 segment of the renal proximal tubule, leading to substantial glucosuria and a reduction in plasma glucose levels. Experimental evidence demonstrated SGLT2 inhibitors may confer neproprotection independently of their glucose-lowering or blood pressure-lowering properties. SGLT2 inhibitors attenuate glomerular hyperfiltration, which is thought to be the initial pathogenic alteration in diabetic and obesity-related CKD: in diabetes, hyperglycemia induces an increase in SGLT2-mediated proximal tubule NaCl reabsorption. The consequent reduction in NaCl distal delivery to the macula densa decreases tubulo-glomerular feedback-mediated afferent arteriolar vasoconstriction, thereby increasing glomerular afferent-to-efferent arteriolar tone, intraglomerular ultrafiltration pressure and GFR (120). Proximal tubule SGLT2 upregulation and increased NaCl reabsorption have been documented in obesity-related CKD, as well, as a result of enhanced sympathetic activity (121,122) and TGF β 1/Smad3 axis activation (123). By virtue of these actions, SGLT2 inhibitors synergize with RAAS inhibitors and their combination may confer incremental renal benefits (124): simultaneous SGLT2–RAAS blockade induces afferent arteriole constriction (SGLT2 inhibition) and efferent arteriole vasodilatation (RAAS blockade), thereby more thoroughly

counteracting early intrarenal haemodynamic abnormalities underlying glomerular hyperfiltration in CKD. Additionally, SGLT2 inhibitors decreased inflammatory and fibrogenic responses, oxidative stress and cell apoptosis in diverse experimental models of CKD(125).

Available data on the impact of SGLT2 inhibitors on CKD derive from analysis of RCTs conducted with efficacy and safety end-points in diabetic population, where SGLT2 inhibitors slowed renal function decline and reduced albuminuria independently of glycemic control (126), while dedicated nephroprotection trials are underway (Evaluation of the effects of Canagliflozin on Renal and Cardiovascular Outcomes in Participants with Diabetic Nephropathy, CREDENCE trial- [clinicaltrials.gov ID: NCT02065791](https://clinicaltrials.gov/ct2/show/study/NCT02065791)).

Beside nephroprotection, SGLT2 inhibitors have also the potential for cardiovascular protection: in the EMPA-REG OUTCOME trial, empagliflozin added on top of standard care was associated with a lower rate of the primary composite outcome of cardiovascular and all-cause mortality (127).

SGLT2 inhibitors may also ameliorate NAFLD: in a post-hoc analysis of a RCT and a small RCT, remogliflozin etabonate and luseogliflozin improved liver fat accumulation and liver enzymes in diabetic patients (128,129). The conclusions of these RCTs are supported by preclinical data, whereby SGLT2 inhibitors prevented diet-induced hepatic steatosis, inflammation and fibrosis, independently of anti-hyperglycemic action (130,131). Potential mechanisms underlying liver-related benefits of these drugs include insulin sensitizing (128), and body fat loss-inducing properties mediated by enhanced lipolysis and fatty acid oxidation(130,131), attenuation of adipose tissue dysfunction and inflammation (132), increased ACE2 activation (133), and intrinsic drug-specific antioxidant and anti-RAGE axis activation properties (134). Furthermore, SGLT2 mRNA expression has also been documented in the liver, where its biological and clinical significance remain unknown (135).

Several issues with SGLT2 inhibitors warrant assessment, including the renoprotective effects in nondiabetic CKD patients, the impact on liver histology, long-term safety in patients with different degrees of renal and hepatic impairment, and the risk of ketoacidosis, which led the FDA to issue a safety warning in 2015.

Conclusions and future perspectives

Despite progress made in the last decade, CKD still remains a major health problem for 2 reasons: it often goes unrecognized and current therapeutic armamentarium has limited effectiveness in retarding disease progression. NAFLD is the most common chronic liver disease and, given the lack of an effective treatment, is becoming the leading indication for liver transplantation in the Western world (5). The shared unmet needs of NAFLD and CKD are therefore boosting research on novel therapeutic targets in each of these 2 conditions

Recent epidemiological data suggest a tight relationship between the presence and severity of NAFLD and the presence and stage of CKD and place NAFLD as an important contributor to the development and progression of CKD, independently of traditional risk factors. When analyzing the pathophysiological basis for this association, striking analogies can be found between fatty liver and CKD: like NAFLD, CKD is characterized by deranged cellular substrate metabolism, ectopic fat deposition, which trigger oxidative stress and inflammatory and profibrotic responses to drive the progression of both disease processes. Our review disclosed a wealth of cellular pathways and mechanisms that represent key contributors to liver and kidney injury and potential therapeutic targets (**Figure 4**). Most of these targets are being currently evaluated in phase II RCTs and some of them, like PPAR- α/δ agonists, FXR agonists and incretin analogues, gave promising results (136). Remarkably, few of them advanced to the same developmental stage in both NAFLD and CKD, reflecting a still low awareness of the similarities in the pathogenic mechanisms underlying these 2 conditions. Beside shared pathogenic mechanisms that promote both liver and kidney injury, the fatty liver may per se promote kidney injury and vice versa, with potentially relevant therapeutic implications: as an example, SGLT2 inhibitors target the renal tubule but may improve both CKD and NAFLD. Whether the relative merits of different therapeutic approaches will translate into a clinical benefit needs assessment in adequately powered, larger RCTs of longer duration with clinical end-points. A key challenge to therapeutic success of these variegated approaches will be the selection of the optimal therapeutic strategy for each patient: NAFLD and CKD progression is likely a

multi-factorial process, involving varied molecular pathways that may operate in different patient subsets and at different stages of disease. Within this context, recent developments in metabolic phenotyping with metabolomics and systems biology technologies will hopefully enable individualized treatment tailored to individual profile.

Given the increasing prevalence of CKD and NAFLD, their direct effects and their acceleration of CVD, strategies to reduce the incidence, progression and complications of these twin plagues are an important priority in healthcare.

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Author Contributions.

GM: conceived and designed the article, undertook literature search and acquired data, critically analyzed the results, drafted the article, gave final approval

GM is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

MC, SC, FDM, SP, FS, RG: undertook literature search and acquired data, critically analyzed the results, contributed to draft of the article, gave final approval

Conflicts of interest.

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FIGURE LEGENDS

Figure 1. Role of nuclear transcription factors in the pathogenesis of NAFLD and CKD.

Each nuclear transcription factor is reported in the textbox at the centre of the scheme and is marked with a superscript number. The different molecular pathways affected by each nuclear transcription factors are written in the boxes surrounding the central box, with the superscript number referring to the corresponding nuclear transcription factor affecting the pathway.

Abbreviations:

HSC: hepatic stellate cell; JNK: c-Jun N-terminal kinase; MAPK: mitogen-activated protein kinase; NOX: NADPH oxidase; Smad: Mothers against decapentaplegic homolog ; VLDLR: VLDL receptor. ER: endoplasmic reticulum; FFA: free fatty acids; FXR: farnesoid X-receptor; GLUT: glucose transporter; MCP-1: monocyte chemotactic protein-1; NLRP3: NOD-like receptor family, pyrin domain containing 3; NOS: nitric oxide synthase; NOX: NADPH oxidase; PGC-1 α : peroxisome proliferator-activated receptor- γ coactivator-1 α ; RAGE: receptor for advanced glycation end-products; ROS: reactive oxygen species; SREBP: sterol-responsive element binding protein; TGF- β : transforming growth factor- β ; VLDL: very low density lipoprotein.

Figure 2: Mechanisms connecting cellular sensors AMP-activated Kinase (AMPK)(panel A), hypoxia-induced factor (HIF)-1 α and mammalian target of rapamycin complex 1 (mTORC1) (panel B) in the pathogenesis of NAFLD and CKD.

In panel B, HIF-1 α and mTORC1 are reported in the textbox at the centre of the scheme and are marked with a superscript number. The different molecular pathways affected by each sensor are written in the boxes surrounding the central box, with the superscript number referring to the corresponding cellular sensor affecting the pathway.

Abbreviations: AMPK: adenosine-monophosphate kinase; EMT: epithelial-to-mesenchymal transition; ER: endoplasmic reticulum; FAS: fatty acid synthase; FFA: free fatty acids; GLUT: glucose transporter; IRS-1: insulin receptor substrate-1; LOXL: Lysyl oxidase-like; MCP-1: monocyte chemotactic protein-1; NO: nitric oxide; NOX: NADPH oxidase; PGC-1 α : peroxisome proliferator-activated receptor- γ coactivator-1 α ; ROS: reactive oxygen species; SREBP: sterol-responsive element binding protein; TGF- β : transforming growth factor- β ; VLDL: very low density lipoprotein;

Figure 3. Role of effectors of redox regulation Apoptosis Signal-Regulating Kinase 1 (ASK1) and Nuclear erythroid 2-related factor 2 (Nrf2) and of galectin-3 in the pathogenesis of NAFLD and CKD. ASK1, Nrf2 and galectin-3 are reported in the textbox at the centre of the scheme and are marked with a superscript number. The different molecular pathways affected by these 3 molecules are written in the boxes surrounding the central box, with the superscript number referring to the corresponding factor affecting the pathway.

Abbreviations: EMT: epithelial-to-mesenchymal transition; HSC: hepatic stellate cell; IRS: insulin receptor substrate; JNK: c-Jun N-terminal kinase; MAPK: mitogen-activated protein kinase; Smad: Mothers against decapentaplegic homolog; Trx: thioredoxin

ABCA1: ATP-binding cassette transporters A1; ACC: acetyl-CoA carboxylase;

Cat: Catalase; Glt-Px: Glutathione peroxidase; Glt-R: Glutathione reductase; *G6PD*: Glucose-6-phosphate 1-dehydrogenase; HPC: hepatic progenitor cells; *TXN-R*: *GST*: Glutathione *S*-transferase, Thioredoxin reductase; *SOD* Superoxide dismutase;; ER: endoplasmic reticulum; FAS: fatty acid synthase; FFA: free fatty acids; FXR: farnesoid X-receptor; GLUT: glucose transporter; MCP-1: monocyte chemotactic protein-1; NOS: NO synthase; NOX: NADPH oxidase; OCA: obeticholic acid; PGC-1 α : peroxisome proliferator-activated receptor- γ coactivator-1 α ; RAGE: receptor for advanced glycation end-products; ROS: reactive oxygen species; SREBP: sterol-responsive element binding protein; TGF- β : transforming growth factor- β .

Figure 4: Molecular pathways mediating the interplay between the liver, kidney, gut and adipose tissue in the pathogenesis of NAFLD and CKD and the effect of their modulation.

Panel 4A: in NAFLD-associated CKD, the gut promotes liver and kidney injury by reduced incretin Glucagon-like Peptide(GLP)-1 and by enhanced microbial production of lipopolysaccharide (LPS) and uremic toxins (URMs), whose excretion is reduced in CKD, further increasing their systemic levels.

Further injurious mechanisms contributing to NASH and CKD at a systemic level include inappropriate activation of Sterol Regulatory Element Binding Protein(SREBP)-2, which promotes intracellular cholesterol accumulation, of cellular sensors Hypoxia Inducible Factor(HIF)-1 and mammalian Target of Rapamycin Complex 1(mTORC1), of oxidative stress-activated Apoptosis Signal-Regulating Kinase 1(ASK1). Collectively, these factors induce insulin resistance(IR), ectopic lipid accumulation which trigger lipoperoxidative stress and enhance secretion of pro-inflammatory cytokines, chemokines CCL2 and CCL5 and of profibrogenic factors like miRNA21 and Galectin-3. The liver, on its side, promotes CKD through enhanced secretion of uric acid and of pro-atherogenic factors including VLDL1 lipoproteins, Cholesterol-Ester Transfer Protein(CETP) and Syndecan-1, which promote atherogenic dyslipidemia, endothelial dysfunction and renal vascular disease. Additionally, in NAFLD there is also an impaired action of hepatokine Fibroblast Growth Factor(FGF)21, whose systemic levels are elevated because of tissue FGF21 resistance and of impaired renal FGF21 excretion in CKD.

Panel 4B: Beside directly antagonizing the action of noxious factors described in panel A, NAFLD and CKD can be ameliorated at intestinal levels by incretin mimetics and by modulating gut microbiota composition with prebiotics, probiotics or synbiotics and at systemic levels by activating cellular energy sensor AMP-activated Protein Kinase(AMPK) and several nuclear transcription factors, including Peroxisome Proliferator-Activated Receptor(PPAR)- α , PPAR- δ and PPAR- γ and Farnesoid X Receptor(FXR) and Nuclear Erythroid 2-related Factor(Nrf2). Please refer to Tables and text for a detailed description of molecular mechanisms underlying gut-liver-kidney connection,

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Table 1. Nutritional factors involved in liver and renal disease in NAFLD and CKD

Dietary fructose	
Cellular effects	Biological effect
<p>Hypothalamus: ↑ dopaminergic tone → ↑ appetite and calorie intake</p>	Weight gain Ectopic fat deposition Insulin resistance inflammation fibrosis Albuminuria Hyperglycemia
<p>Hepatocyte, skeletal miocyte: ↓ FFA oxidation and RE</p>	
<p>Adipocyte: Adipose tissue dysfunction → ↓ adiponectin secretion</p>	
<p>Liver and kidney cells(uric acid-mediated): NLRP3 inflammasome activation → ↑ IL-1 secretion → ↑ macrophage accumulation ↓ AMPK activity → ↓ FFA oxidation SREBP-1c activation → ↑ <i>de novo</i> lipogenesis NOX activation → ROS generation → podocyte loss, tubule cells EMT, endothelial injury</p>	
<p>Pancreatic β-cell: ↓ postprandial insulin response</p>	
vitamin D deficiency	
Cellular effects	Biological effect
<p>Skeletal miocyte: ↓ IRS-1 activity → ↓ insulin signaling</p>	Insulin resistance, Ectopic fat
<p>Hepatocyte, Kupffer cell: ↑ TLR-2/4/9 expression → ↑ sensitivity to LPS and FFA-induced inflammatory cytokines ↑ ROS production → ↑ hepatocyte apoptosis, ↑ inflammatory cell recruitment</p>	

<p>Hepatic stellate cell:</p> <p>VDR down-regulation → ↑ TGF-β secretion and SMAD2 activation → ↑ fibrogenesis</p>	<p>deposition</p> <p>Inflammation</p>
<p>Glomerular podocyte:</p> <p>↑ p38- and ERK-mediated apoptosis → ↑ podocyte injury → glomerular barrier disruption</p> <p>↑ RAS activation → glomerulosclerosis</p>	<p>Hepatic</p> <p>fibrosis</p> <p>proteinuria</p>
<p>Renal tubule cell and interstitial macrophage:</p> <p>↑ SREBP-1c/SREBP-2 → ↑ toxic cholesterol and FFA lipid accumulation</p> <p>↓ FXR/PPAR-α activation → ↑ toxic cholesterol and FFA lipid accumulation</p> <p>↑ NF-κB pathway activation → ↑ inflammation and macrophage accumulation</p> <p>↑ TGF-β /Wnt pathway activation → tubule cell EMT → ↑ fibrogenesis</p>	<p>ectopic fat</p> <p>deposition</p> <p>tubulo-</p> <p>interstitial</p> <p>inflammation</p> <p>and fibrosis</p>

Abbreviations: AMP: adenosine-monophosphate; AMPD: AMP deaminase; AMPK: adenosine-monophosphate kinase; ATP: adenosine triphosphate; EMT: epithelial-to-mesenchymal transition; CYP: cytochrome protein; ER: endoplasmic reticulum; ERK: extracellular signal-regulated kinase;; FFA: free fatty acids; FXR: farnesoid X-receptor; HSC: hepatic stellate cell; IL: interleukin; IRS-1: insulin receptor substrate-1; LPS: lipopolysaccharide; MCP-1: monocyte chemotactic protein-1; NF-κB: nuclear factor-κB; NLRP3: NOD-like receptor superfamily, pyrin domain containing 3; NO: nitric oxide; NOX: NADPH oxidase PPAR: peroxisome proliferators-activated receptor; RAS: rennin-angiotensin system; REE: resting energy expenditure; ROS: reactive oxygen species; SHP: small heterodimer partner; SMAD. small mother against decapentaplegic; SOCS-3: suppressor of cytokine signaling 3; SREBP: sterol-responsive element binding protein; TGF-β: transforming growth factor- β; TLR: toll-like receptor; TNF: tumor necrosis factor; VDR; vitamin D receptor;

Table 2. Mechanisms of action and developmental stages of drugs targeting both NAFLD and CKD

Mechanism of action	Molecule	Developmental stage	
		NAFLD	CKD
Xanthine oxidase inhibitors	Allopurinol, febuxostat	Preclinical	Iia CKD-FIX (clinicaltrials.gov ID: NCT12611000791932) FEATHER (UMIN ID: UMIN000008343)
Vitamin D supplementation	Natural (ergocalciferol, cholecalciferol, calcitriol), Synthetic VDR agonists(doxercalciferol, paricalcitol)	Iia Clinicaltrials.gov ID: NCT02098317.	Iia Clinicaltrials.gov ID: NCT00893451, NCT01623024
PPAR-δ agonists	GW0742	-	Preclinical (25)
	GW610742	-	Preclinical(26)
PPAR-α/δ agonists	GFT505	Iib GOLDEN-505(27)	-
PPAR- α/γ agonists	Saroglitazar	Iia (CTRI no.: CTRI/2010/091/000108	-
	Aleglitazar	-	Iib ((32)
SREBP-2 antagonists	Natural antioxidants(myricetin)	-	Preclinical (34)
FXR agonists	Obeticholic acid	Iia FLINT(39)	Preclinical (36,37)
miRNA-21	Antagomir-21	Preclinical(42)	Preclinical(43)

antagonists			
AMPK activators	Natural: curcumin berberine, monascin ankaflavin	Preclinical(45,46)	Preclinical (47)
	Synthetic: oltipraz	Ia(clinicaltrials.gov ID: NCT01373554).	-
HIF-1α inhibitors	YC-1, AC, POC	Preclinical (53)	-
Dual mTORC1/2 inhibitor	Rapamicin	Preclinical(55)	Preclinical(56)
ASK1 inhibitor	GS-4997	Ia (ClinicalTrials.gov ID: NCT02466516)	Ia(ClinicalTrials.gov ID: NCT02177786)
Nrf2 activators	Oltipraz , Bardoxolone methyl	Ia(clinicaltrials.gov ID: NCT01373554)	(clinicaltrials.gov ID: NCT02316821)
CCR2 receptor antagonist	CCX140-B	-	Ia(74)
CCR2/5 receptor antagonist	Cenicriviroc	Ia: CENTAUR (ClinicalTrials.gov ID: NCT02217475)	-
	BMS-813160 PF-04634817	-	Ia (ClinicalTrials.gov ID: NCT01752985, NCT01712061)
Galectin-3 inhibitors	GM-CT-01	Preclinical(84)	-
	GR-MD-02	Phase I (ClinicalTrials.gov ID: NCT01899859)	-
	GCS-100	-	Phase Ia: ClinicalTrials.gov ID:

			NCT01843790
Incretin-based therapies	Incretin mimetics: liraglutide, exenatide	Iia LEAN(84)	Iia(86,87)
	DPP-4 inhibitors: Saxagliptin, linagliptin	Preclinical(42)	Iia (MARLINA-T2D, CARMELINA)
Gut microbiota manipulation	Prebiotics, probiotics, synbiotics	Iia(101)	Iia(Australian New Zealand Clinical Trials Registry Number: ACTRN12613000493741)
CETP inhibitors	Fc-CETP6	Preclinical(108)	-
Inhibitors of syndecan-1 shedding	sphingosine-1-phosphate	-	Preclinical(116)
FGF-21 analogs	PEG-FGF21, recombinant FGF21, anti-FGFR1 mAb	Iia(121)	-
SGLT2 inhibitors	remogliflozin etabonate, luseogliflozin, ipragliflozin	Iia(130,131)	-
	dapagliflozin, canagliflozin, empagliflozin	-	Iia(134, CREDENCE trial)

Abbreviations: AC: 1-Adamantaneformoxyl-3-(5'-hydroxymethyl-2'-furyl)indazole; .FGFR. FGF-21 receptor; GM-CT-01: galactomannan; GR-MD-02: galactoarabino-rhamnogalaturonan; PEG: pegylated; mAb: monoclonal antibodies; YC-1: 1-Benzyl-3-(substituted aryl)-5-methylfuro[3,2-c]pyrazole

Table 3 Role of incretin-based therapies and gut microbiota in the pathogenesis of NAFLD and CKD.

Incretin analogues: GLP-1 receptor agonists

Cellular mechanism	Biological effect
Hypothalamus: ↓ appetite	↓ calorie intake
Skeletal miocyte: ↑ Glucose uptake ↑ Glycogen synthesis	↑ insulin sensitivity
Hepatocyte: ↑ Glycogen synthesis and ↓ Gluconeogenesis ↑ Autophagy ↑ cAMP→↑AMPK and SIRT-1 activity ↓ FGF-21 secretion	↑ insulin sensitivity ↓ hepatic steatosis
Adipocyte: ↑ Lipolysis ↑ Glucose uptake ↑ Lipogenesis (↑ FFA synthesis, uptake and reesterification, ↑ LPL activity) ↑ Adiponectin secretion	↑ insulin sensitivity ↓ adipose tissue dysfunction
Proximal tubule cell: ↓ NHE3 activity→↑ Na-uresis →↓ Na hyperreabsorption ↑ PPAR-α→↑ FFA oxidation Glomerular endothelial cell, mesangial cell, tubule cell: ↓ apoptosis ↓ AGE/RAGE-axis activation ↓ IL-1/MCP-1/TGF-β secretion→↓ monocyte recruitment and fibrogenesis ↑ NO synthase activity ↑ cAMP→NOX down-regulation→↓ ROS generation ↓ AngII activity→↓ IRS-1 phosphorylation → ↑ IRS-1 signalling	↑ Na delivery to distal tubule→ ↓ tubulo-glomerular feed-back→ ↓ glomerular hyperfiltration ↓ blood pressure ↓ fat infiltration ↓ glomerular sclerosis ↓ inflammation and fibrosis ↓ endothelial dysfunction ↑ insulin sensitivity
Incretin analogues: DPP-4 inhibitors	
Liver, kidney: ↑ GLP-1 activity	Same effects of GLP-1R

<p>Kidney: ↑ BNP → ↑ natriuresis and vasodilation, ↓ RAS and sympathetic activity ↑ NPY and PYY → ↑ AngII-mediated vasoconstriction ↑ SDF-1a → ↑ mesenchymal stem cells recruitment</p>	<p>agonists</p> <p>Ultimate effect depends on local tissue activity of different substrates inactivated by DPP-4</p>
<p>Altered gut microbiota composition and intestinal barrier disruption</p>	
<p>Cellular mechanism</p>	<p>Biological effect</p>
<p>Hepatocyte, macrophage, HSC, renal vascular endothelial cell, podocytes : ↑ LPS-TLR-4 axis activation → ↑ proinflammatory cytokines/TGF-β ↑ LPS-TLR-4 axis activation → ↑ NOX type II activity → ROS production</p>	<p>hepatic and renal inflammation hepatic and renal fibrogenesis endothelial dysfunction</p>
<p>Enterocyte: ↓ SCFA production → ↑ epithelial injury and ↓ GLP-1 secretion</p>	<p>gut barrier disruption</p>
<p>Hepatocyte: ↑ conversion of intestinal TMA to trimethylamine-N-oxide (TMAO) by flavin-containing monooxygenases</p>	<p>Hepatic steatosis Hepatic necroinflammation</p>
<p>Tubule cells: ↑ TMAO-induced TGF-β/SMAD-3 axis activation</p>	<p>atherosclerosis tubule-interstitial fibrosis</p>
<p>Skeletal miocyte: ↑ URM-induced phosphorylation of IRS-1 → ↓ IRS-1 signalling</p>	<p>Insulin resistance</p>
<p>Hepatocyte: ↑ URM-induced <i>de novo</i> lipogenesis ↓ URM-induced VLDL secretion</p>	<p>Insulin resistance Hepatic steatosis</p>
<p>Adipocyte: ↑ URM-induced leptin secretion ↓ URM-induced <i>de novo</i> lipogenesis ↑ URM-induced Zinc-α2-glycoprotein (ZAG) and ↓ perilipin expression → ↑ lipolysis</p> <p>Macrophage:</p>	<p>Insulin resistance Adipose tissue dysfunction Adipose tissue and systemic inflammation</p>

Abbreviations: AGE: advanced glycation end-products; AMP: adenosine-monophosphate; AMPK: adenosine-monophosphate kinase; AngII: angiotensin II; BNP: brain-derived natriuretic peptide; cAMP: cyclic adenosine-monophosphate; DPP-4: dipeptidyl peptidase protein-4; ERK: extracellular signal-regulated kinase; FFA: free fatty acids; FGF: fibroblast growth factor; GLP-1: glucagon-like peptide-1; IL: interleukin; IRS-1: insulin receptor substrate-1; LPL: lipoprotein lipase; LPS: lipopolysaccharide; MCP-1: monocyte chemoattractant protein-1; NHE3: Na⁺-H⁺ exchanger 3; NF-κB: nuclear factor-κB; NO: nitric oxide; NOX: NADPH oxidase; NPY: neuropeptide Y; PPAR: peroxisome proliferator-activated receptor; PYY: peptide YY; RAAS: renin-angiotensin-aldosterone system; ROS: reactive oxygen species; SDF-1a: stromal-derived Factor; SIRT: sirtuin; TGF-β: transforming growth factor- β; TLR: toll-like receptor; TMA: trimethylamine; TNF: tumor necrosis factor; VLDL: very low density lipoprotein.

Figure 1

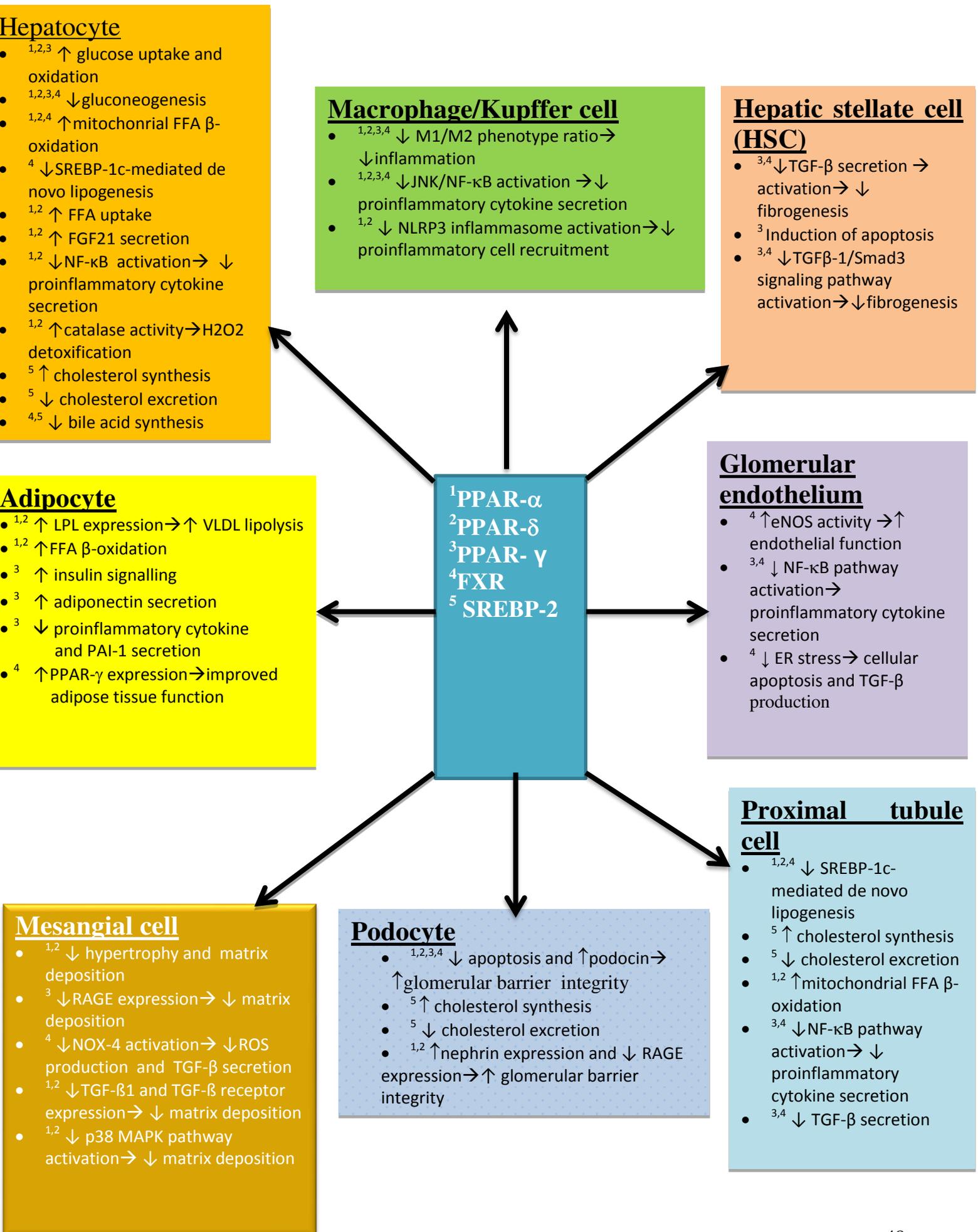


Figure 2 Panel A

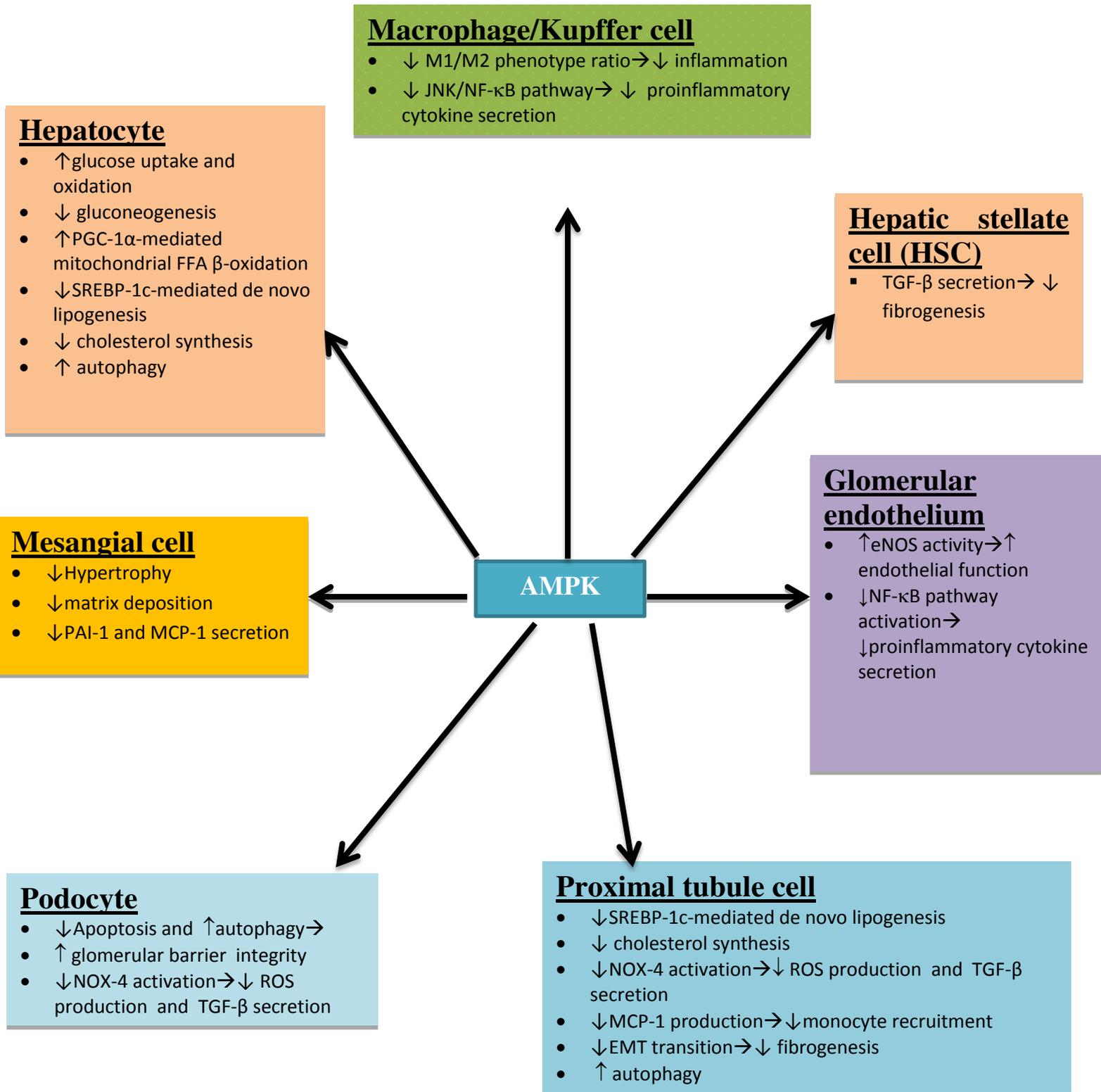


Figure 2 Panel B

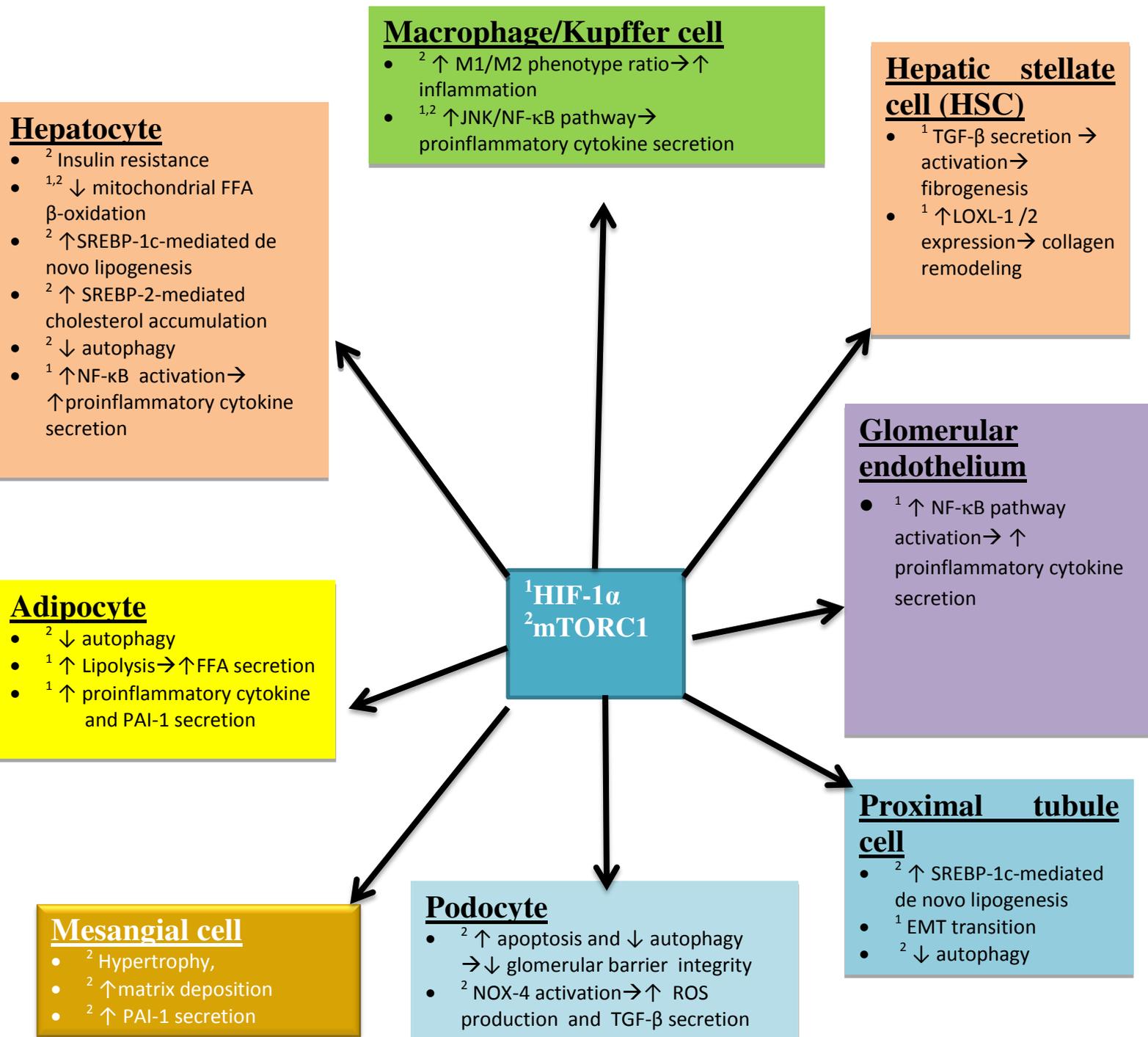


Figure 3

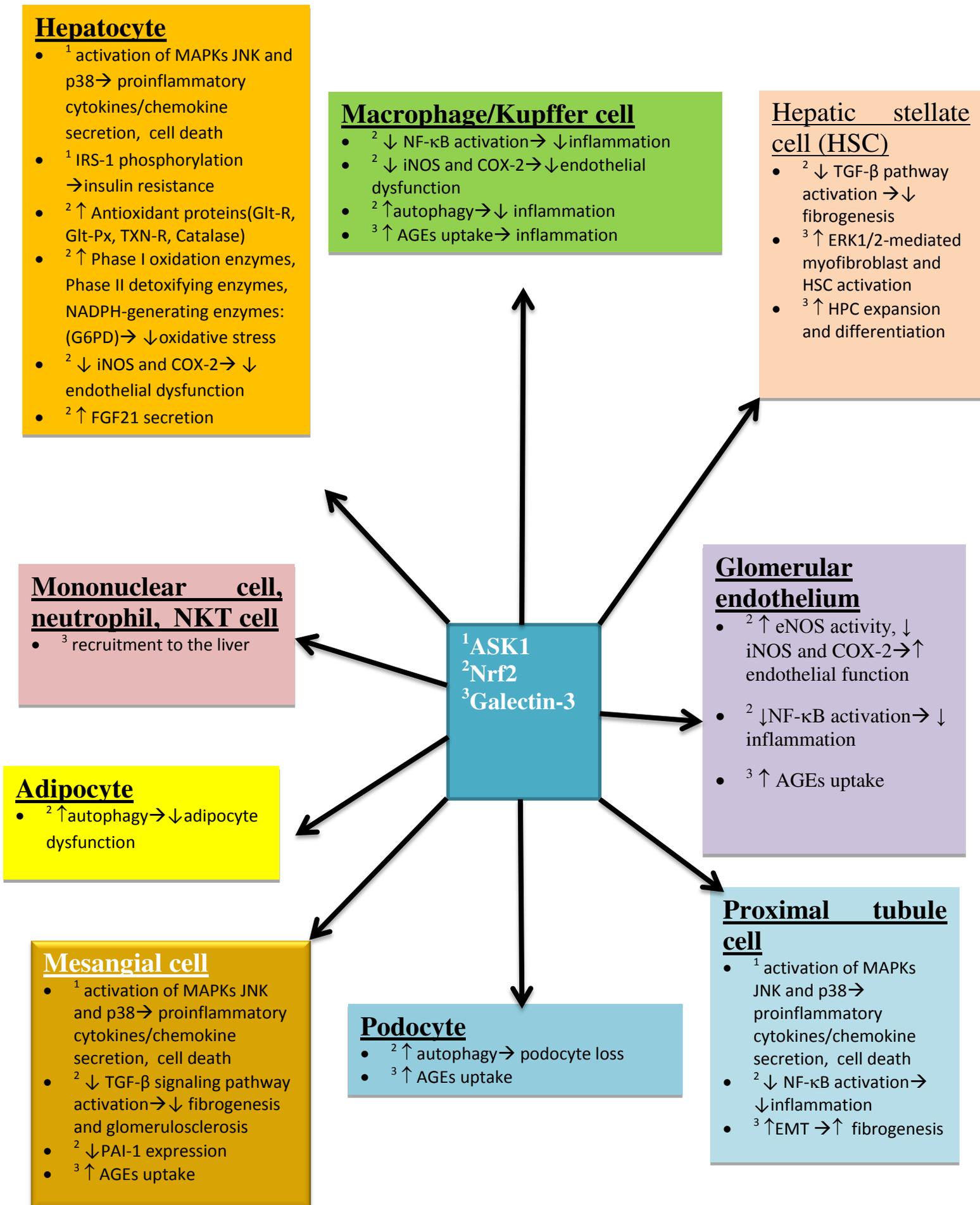
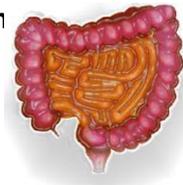


Figure 4A

↓GLP-1 production
↑ URM's production
↑ LPS production

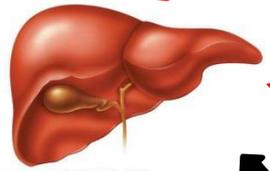


↑ IR
↓ FFA Oxidation
↑ Chol Accumulation
↑ Oxidative Stress
↑ Pro-Inflammatory Cytokines/
Chemokines (CCI2, CCL5)
↑ Galectin-3/miRNA21 → Fibrogenesis

miRNA21
SREBP-2
ASK1

↑ VLDL1/CETP/Syndecan-1/uric acid secretion
↓ FGF21 action

HIF
mTORC1



↑ IR
↓ FFA Oxidation
↑ Chol Accumulation
↑ Oxidative Stress
↑ Pro-Inflammatory Cytokines/
Chemokines (CCI2, CCL5)
↑ Galectin-3/miRNA21 → ↑ Fibrogenesis

↑ IR
↑ De novo Lipogenesis
↑ Pro-Inflammatory Cytokines /Chemokines(CCL2/CCL5)

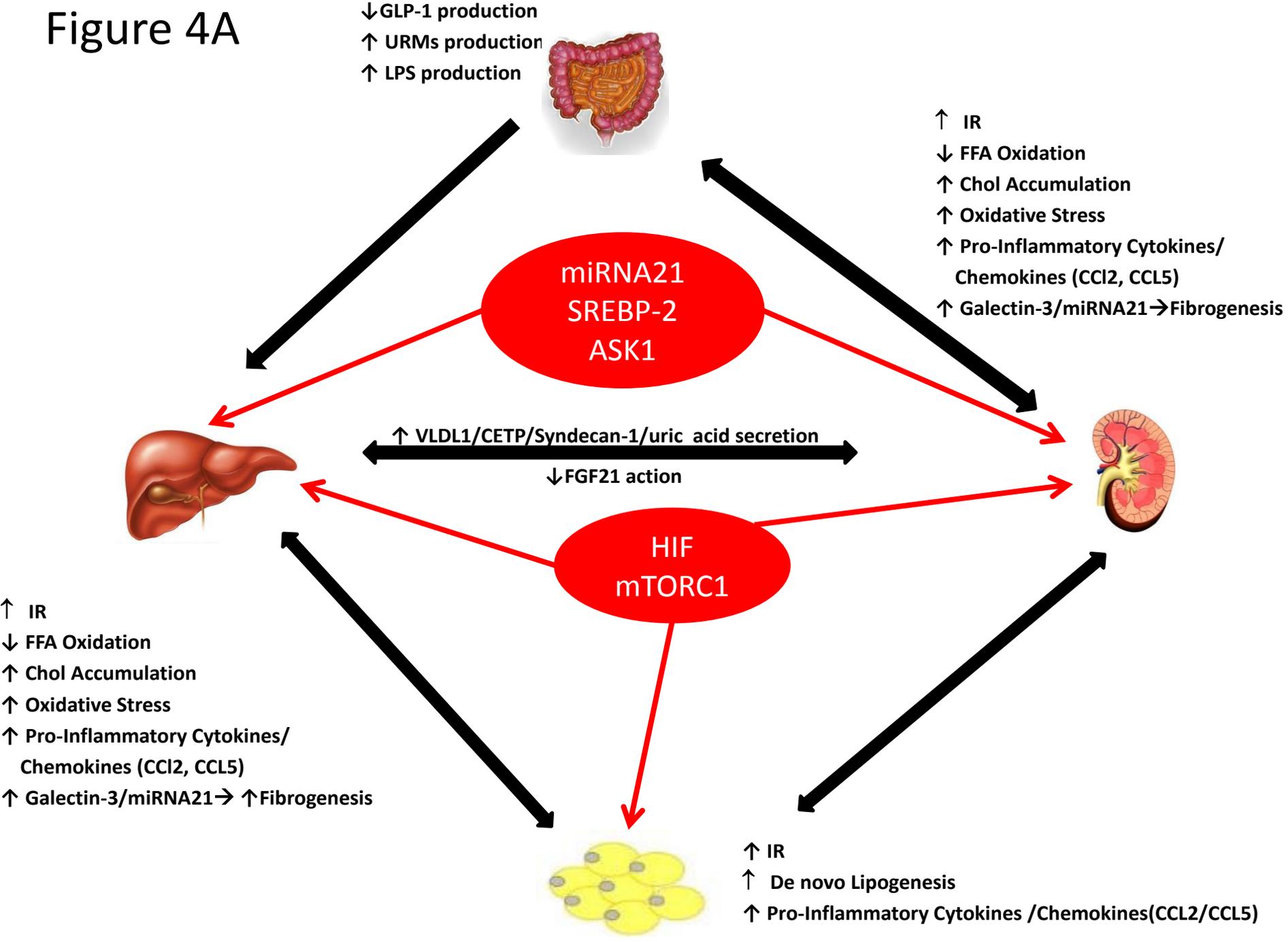
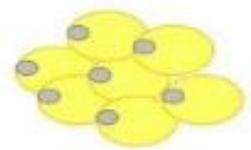


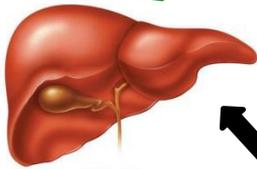
Figure 4B

Incretin mimetics
Prebiotics, probiotics
Synbiotics



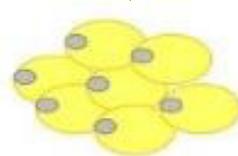
- ↓ IR
- ↑ FFA Oxidation
- ↓ Chol Accumulation
- ↓ Oxidative Stress
- ↓ Pro-Inflammatory Cytokines/Chemokines
- ↓ galectin-3/miRNA21 → ↓ fibrogenesis

PPAR- α
AMPK
Nrf2



PPAR- δ
PPAR- γ
FXR

- ↓ IR
- ↑ FFA Oxidation
- ↓ Chol Accumulation
- ↓ Oxidative Stress
- ↓ Pro-Inflammatory Cytokines and Chemokines
- ↓ galectin-3/miRNA21 → ↓ fibrogenesis



- ↓ IR
- ↓ De novo Lipogenesis
- ↓ Pro-Inflammatory Cytokines Production

