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Comparison and Contrast of Human and Companion Animal Metabolic Lipid Hepatopathies: NAFLD/NASH Versus the Feline Hepatic Lipidosis Syndrome

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Glossary of Abbreviations
ACC: Acetyl-CoA Carboxylase
AMPK: Adenosine Monophosphate-Activated Protein Kinase
ATGL: Adipose Triglyceride Lipase
FAS: Fatty Acid Synthase
FABPs: Fatty Acid Binding Proteins
FHL: Feline Hepatic Lipidosis Syndrome
HDL-C: High-Density Lipoprotein Cholesterol
HSL: Hormone-Sensitive Lipase
LCFA: Long-Chain Fatty Acids
LDL-C: Low-Density Lipoprotein Cholesterol
MCP-1: Monocyte-Chemoattractant Protein-1
MeTS: Metabolic Syndrome
MGL: Monoglycerol Lipase
NAFLD: Non-Alcoholic Fatty Liver Disease
NASH: Non-Alcholic Steatophepatitis
PKA: Protein Kinase A
PPAR-α: Peroxisome Proliferator Receptor-Alpha
PPAR-γ: Peroxisome Proliferator Receptor-γ
RAS: Renin-Angiotensin System
SAT: Subcutaneous Fat
SIBO: Small Intestinal Bowel Overgrowth
TGH: Triglycerol Hydrolase
WAT: White Adipose Tissue

Introduction

This discussion has two aims: 1) to summarize current understanding of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in humans, the most common nonviral forms of liver disease in humans with proven association to metabolic syndrome (MeTS); and 2) to enable comparison of the metabolic dysregulation in obese cats with feline hepatic lipidosis (FHL) syndrome. Data regarding clinical features of FHL is derived from 188 cases managed in the author's hospital.

The Metabolic Syndrome

Central to the diagnosis of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) is diagnosis of MeTS. Initially characterized in 1988 by Reaven,[1] MeTS unites four major physiologic conditions: 1) insulin resistance, 2) systemic hypertension, 3) dyslipidemia (increased triglyceride, reduced high-density lipoprotein cholesterol [HDL-C] and sometimes moderately increased low-density lipoprotein cholesterol [LDL-C] levels), and 4) essential hypertension (Figure 1). Although these associations are well-established, their pathophysiological basis is complex and there are no simple unifying features. While central or visceral obesity is considered a phenotypic characteristic, some researchers argue that this represents diagnostic artifact-lacking prognostic or therapeutic value.

Proponents argue that "central" or "visceral" adiposity reflects abnormal distribution of "metabolically unique" fat (visceral white fat [VAT]) that correlates with insulin resistance. Abundance of VAT, having some metabolic characteristics resembling brown fat, can be quantified using magnetic resonance spectroscopy/imaging (MRS/I) allowing noninvasive diagnostic surveillance. Insulin

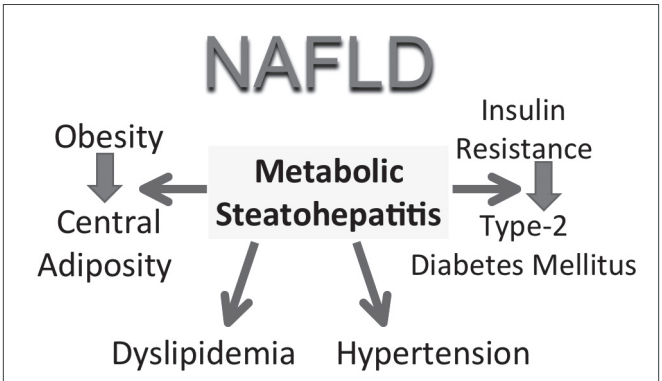


Figure 1

resistance as a qualifying criteria of MeTS has been problematic in application as it cannot be precisely defined or measured.[2] Adding confusion to the understanding of MeTS is that despite a large body of published work, controversy remains regarding its clinical diagnostic utility.[3,4] What is agreed upon is that obesity predicts metabolic predisposition.

Non-Alcoholic Fatty Liver Disease (NAFLD)/Non-Alcoholic Steatohepatitis (NASH)

The pathologic union between VAT and the liver may reflect their common embryologic origin.[6] Each is comprised of highly metabolic cells (adipocytes or hepatocytes) located in proximity to and having cross talk with reactive cell populations (e.g., immune cells such as NK and NKT cells, Kupffer cells, macrophages, hepatic stellate cells, and endothelial cells). In addition, each system has intimate access to a substantial vascular network. Further, the tissue organization of liver and adipose provides anatomic configurations ideal for continuous dynamic interaction between immune and metabolic responses.[7] The cellular interface in adipose is emerging as an important factor in both initiation and development of the metabolic disturbances, linking obesity with type-2 diabetes in humans and some animal models.[6,8,9]

NAFLD is a condition in which hepatocytes contain supra-physiological amounts of fat. Histopathologically, it can appear as simple steatosis (NAFL) or can be associated with an inflammatory reaction defined as NASH (Figures 2 and 3). NASH may exist with or without portal fibrosis and may lead to fatty liver-associated cirrhosis (NASH-induced cirrhosis). Approximately 10% of NAFLD patients develop NASH with an estimated 8% to 26% of individuals, depending on the study cited, progressing to cirrhosis.[6,10] NASH appears to increase the risk for hepatocellular carcinoma (HCC), portal hypertension (due to hepatic fibrosis) associated with gastroesophageal bleeding (acquired portosystemic shunts), ascites, and liver failure.[6]

Non-Alcoholic Fatty Liver Disease (NAFLD)

As defined, NAFLD is characterized by increased hepatocyte triglyceride (TG) vacuolation but lacks necroinflammatory histologic features. Overnutrition is considered the first step in its development with surfeit energy fostered by physical inactivity and ingestion of certain nutrient excesses (i.e., saturated fat and fructose). In health, adipocytes, rather than the liver, physiologically store fat. Peripheral subcutaneous adipose tissue (SAT), composed mostly of small, differentiated, insulin-sensitive adipocytes, absorb postprandial free fatty acids (FFA) and lipoprotein-bound TG. In adipocytes, TG lipogenesis, and their storage in lipid bodies surrounded by a monolayer of lipase-regulating proteins, is maintained until energy is needed during the interdigestive interval or fasting. SAT adipocytes secrete adiponectin that normally protects the liver from steatosis, increasing long-chain FFA β-oxidation and suppressing hepatic lipogenesis. The MeTS associates with failure of SAT to efficiently store fat, leading to adipocyte hyperplasia and de-differentiation as they continue to release FFA from TG (lipolysis). Adipocyte de-differentiation and recruited macrophages, which release TNF-α, suppress expression and secretion of adiponectin. Insulin resistance (hyperinsulinemia and hyperglycemia) related to unrestricted release of FFA, downregulated adiponectin, increased leptin, and TNF-α release, in addition to a considerable number of metabolic and hormonal interactions, stimulate hepatic FA synthesis through effects on transcription factors.

Normal liver contains ~5% fat (as TG) as quantified by MRS/I (98% sensitivity for steatosis detection). Hepatic TG levels increase up to tenfold normal in NAFLD/NASH with lipid droplets contained within

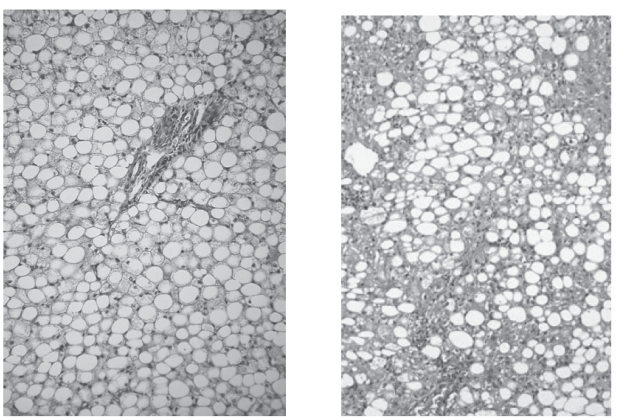


Figure 2

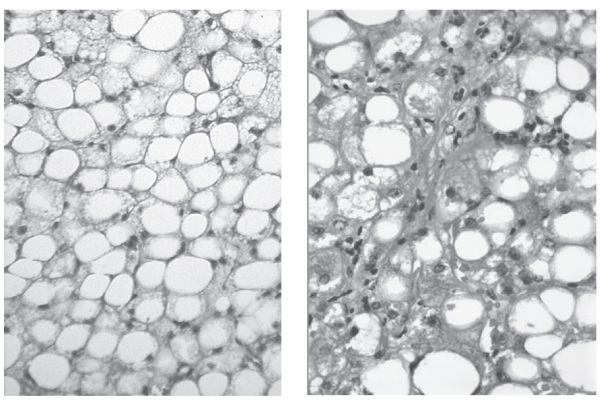


Figure 3

membrane bound vacuoles (endoplasmic reticulum) as macrovesicular (large) cytosolic inclusions. However, FFA, diacylglycerol, toxic phospholipids and cholesterol-esters are considered the most tissue damaging and pro-inflammatory (lipotoxic) in NAFLD/NASH, rather than TG.

NAFLD has a prevalence ranging from 16% to 33% in different ethnic populations (14% to 16% in Asians, 31% to 33% in African-Americans and Caucasians, and 45% among Hispanics). Depending on the study, NASH develops in up to 33% of these (some estimates predict NASH affects only 3% of patients with NAFLD, 20% to 30% of obese patients and diabetics; and 35% to 40% of morbidly obese individuals).[11-14] Different prevalence rates partially coordinate with ethnic tendencies for visceral adiposity. In Western cultures, NAFLD is one of the most common causes of increased liver enzyme activity (particularly GGT & ALT), ultrasonographic hepatic hyperechogenicity and/or hepatomegaly, abdominal discomfort and chronic fatigue. However, up to 75% of affected individuals have normal serum enzymes. Metabolic disorders increase risk with 67% to 70% of obese and diabetic patients and up to 96% of morbidly obesity subjects developing NAFLD.[10]

Diagnosis of NAFLD is based on an absence of excessive alcohol intake (defined by two standard drinks [20g ethanol] daily for men and one standard drink [10g ethanol] daily for women).[15] Categorized as "primary" or "secondary" depending on the presence of antecedent or co-morbid disorders, primary NAFLD reflects concurrent MeTS and insulin resistance. Secondary NAFLD emerges in association with infection (viral), drug therapies, parenteral feeding or rare metabolic and congenital disorders (Table 1).[6] One study characterized a 4.6-fold risk for NAFLD in obese humans with a 5.8-fold risk in obese humans regularly consuming alcohol ("heavy drinkers").[6] So, while NAFLD may affiliate with a more primary condition, there is no single consistent underlying cause and nearly universal risk in obese individuals (BMI>30 kg/m2 in Europeans, >25 kg/m2 in Asians). Because NAFLD commonly associates with features of MeTS, it has recently been retermed metabolic steatohepatitis.[11]

Table 1: Differential Diagnosis of Non-Alcoholic Fatty Liver Disease (NAFLD) in Humans Adapted from reference 12

Type	Pathophysiological Association	
Primary	Obesity	
	Insulin resistance/Metabolic Syndrome	
	Diabetes Mellitus (I/II)	
	Dyslipidemia	
Secondary		
Viral	Hepatitis B, C, Epstein Barr	
Autoimmune	Autoimmune hepatitis, SLE	
Genetic Disorders	Wilson's Disease (Cu storage)	
	Hemochromatosis (Fe storage)	
	Alpha 1 antitrypsin deficiency	
	Celiac disease	
Drugs	Amiodarone	Tamoxifen
	Corticosteroids	Methotrexate
	Ca Channel Blockers	Valproate
	Anti-retroviral agents	
Toxins	Organic solvents	
	Mushroom toxicity	
Lipid Disorders	Hypobetalipoproteinemia	
	Lipodystrophy	
Metabolic Disorders	Reyes Syndrome (microvesicular hepatic lipidosis)	
	Weber-Christian Syndrome (panniculitis)	
	Pregnancy	
Endocrine Disorders	Hypopituitarism	
	Cushing's Syndrome	
	Hypothyroidism	
Nutritional	Total Parenteral Nutrition	
	Rapid Weight Loss	
	Starvation	
	Jejuno-ileal bypass	

Non-Alcoholic Steatohepatitis (NASH)

Non-alcoholic steatohepatitis is the necroinflammatory, profibrogenic form of NAFLD that can lead to cirrhosis, inextricably linked with type-2 diabetes and MeTS. A diagnosis of NASH is reserved for histologically confirmed cases.[16,17] While most common in obese patients, there are "metabolically obese" normal weight individuals who also exhibit NAFLD/NASH. These patients have a history of recent or progressive weight gain or rapid weight loss; however, the more severe forms of NASH associate with obesity. While NASH is histologically characterized by hepatic steatosis, hepatocyte ballooning, nonsuppurative inflammatory cell infiltrates (lobular inflammation) and Mallory-Denk bodies, with or without fibrosis, universal characterization has been challenging because of the many independent morphologic features (Figures 2 and 3).

Histologic classification is now regimented using a rigorous histopathologic grading scheme.[15-18] Multiple pathologists naively and independently graded diagnostic features on 50 anonymous biopsy samples with each individual grading the same tissues twice (naively) against a list of morphologic criteria. Kappa statistics characterizing agreement among individuals and repeatability of assigned scores by the same individuals identified characteristics with semiquantitative assessment value.[17] In non-cirrhotic livers, steatosis is a necessary criteria and considered sufficient for histologic diagnosis

of NAFLD. While steatosis is a necessary prerequisite of NASH, this diagnosis requires lobular inflammation and hepatocellular ballooning degeneration. However, a NASH-cirrhotic liver may lack evidence of antecedent steatosis or steatohepatitis owing to architectural reorganization, parenchymal loss and fibrosis, confusing retrospective clinical studies.

Hepatocellular ballooning in NASH, defined on hematoxylin and eosin (H&E) staining, appears as 1.5- to 2-fold enlargement of hepatocytes with "rarefied" cytoplasm.[16-19] Ultrastructural and immunohistochemical studies confirm that TG fat droplets associate with the endoplasmic reticulum, cytoskeletal injury evidenced by cytoplasmic keratin 18 depletion, and Mallory-Denk bodies (keratin 8> keratin 18). Immunohistochemical studies have confirmed that ballooned hepatocytes contain oxidized phosphatidylcholine in the phospholipid-rich rim of fat droplets and altered expression of fat droplet associated proteins (PAT family) known to regulate insulin-sensitive droplet lipase activity.[20]

Type of Lipid Vacuolation & Zonal Distribution Hepatocellular Triglyceride in NAFLD/NASH

The most common form of steatosis in NAFLD/NASH is macrovesicular vacuolation with a single or several large TG filled vacuoles displacing cytoplasm and the nucleus of the hepatocyte to the cell periphery. In some cases, a mixture of small and large droplets associates. True microvesicular steatosis has nearly unappreciable or uncountable droplets, creating a foamy cytoplasmic appearance with a centrally retained nucleus. A recent study defined microvesicular steatosis in up to 10% of NAFLD/NASH cases. However, it is never the predominant form of vacuolation and usually occurs in a non-zonal distribution in patches of contiguous hepatocytes.[21] Hepatocytes with microvesicular lipidosis often contain megamitochondria (identified on routine light microscopy H&E staining). Microvesicular steatosis is far more common in other unique syndromes, such as acute fatty liver of pregnancy and other processes associated with compromised mitochondrial β-oxidation. The more typical large fat droplets are surmised to represent fusion of small droplets initially formed on the surface of the endoplasmic reticulum in NAFLD.[21] Small lipid droplets have a limiting phospholipid membrane implicated as the site of initial oxidative stress in generation of the NASH lesion.[20]

Zonal distribution of hepatocyte vacuolation in NAFLD is variable, but most commonly involves Zone 3 (periacinar or perivenular). Zone 3 steatosis predominates in adult NAFLD, alcoholic steatosis or steatohepatitis, steatosis secondary to drug toxicity, and inborn metabolic abnormalities (lipodystrophy). Zone 1 distribution predominates in pediatric NAFLD, hepatitic C associated NAFLD, cachexia, protein-calorie malnutrition (kwashiorkor), AIDs, TPN, cystic fibrosis, phosphorous poisoning, corticosteroids (in humans), and amiodarone toxicity. Pediatric and occasionally adult NAFLD/NASH may also have a panacinar (diffuse) or azonal (nonuniform or random) distribution.

Pattern and Type of Inflammation/Necrosis NASH

Necroinflammatory lesions in NASH are either lobular and portal or one of these individually. Lobular infiltrates may involve a neutrophilic or mononuclear infiltrate (lymphocytes, monocytes, plasma cells and eosinophils) or only the latter (Figure 3).[17,18] Portal inflammation is usually mononuclear ± lipogranulomas and is most notable in three NASH settings: pediatric or resolution of NASH after treatment and with severe steatohepatitis in adults or children. Frequently overlooked in NASH are increased Kupffer cell aggregates in Zone 3, indicating loss of their typical Zone 1 predominance.[16] In some cases, Zone 3 inflammation is so intense that it confuses acinar architecture. Several studies have shown that oxidized hepatocellular membranes and antibody formation against oxidized membrane components affiliate with mononuclear inflammation. Lipogranulomas may develop in Zone 1 or Zone 3 or randomly; zonal lesions are associated with fibroinflammatory lesions. Lobular or random lipogranulomas usually involve single droplet macrovesicular steatosis with a damaged or dying hepatocyte surrounded by a lipopigment-containing Kupffer cell. Accumulation of lipofuscin pigment in Zone 3 hepatocytes represents previous oxidative membrane damage. Hepatocyte injury and death are represented by ballooning degeneration, acidophil bodies (apoptotic hepatocytes with eosinophilic condensed cytoplasm and karyolytic remnants) or random foci of necrosis. Random necrotic foci represent cytolytic necrosis and associate with small focal inflammatory infiltrates. Perisinusoidal fibrosis in a "chicken wire pattern" frequently associates with Zone 3 ballooning degeneration where lobular collapse intermixes with inflammation.[16,17] Fibrosis may develop in Zone 1 or Zone 3 and progresses to bridging within or between zones. Cirrhosis is the final stage and is estimated to evolve in ~20% to 25% of NASH cases.

Clinicopathologic and Imaging Features of NAFLD/NASH

Remarkably, hepatic aminotransferase activity is seldom more than fivefold the upper limit of normal and typically fluctuates; normal values being observed in >2/3 of NASH patients at any time.[22,23] Liver enzyme activity, total bilirubin concentrations and other routinely used markers for identification of acquired liver disease

or dysfunction are normal in nearly 50% of obese patients with NAFLD or severe NASH. Thus, humans with focal steatosis cannot be differentiated from patients with NAFLD/NASH based on clinicopathologic features.[23-25] Hepatic ultrasonography typically discloses hyperechoic hepatic parenchyma and, in some patients, subjective hepatomegaly. Thus, histologic evaluation of tissue is essential for accurate diagnosis. Yet, considerable problems have complicated clinical utility of needle biopsy samples, as in other forms of liver disease. Needle samples are known to inconsistently disclose diagnostic features, may fail to disclose fibrosis, and since they are usually collected from a single lobe, may not disclose representative lesions. Biopsy diagnoses are also complicated by the cyclic nature of NAFLD/NASH lesions. Liver enzyme activity and steatosis spontaneously fluctuate, often improving as necroinflammation and fibrosis progress.

Pathophysiology of NALD and NAFLD/NASH

NAFLD/NASH has no single cause, lacks consistent clinicopathological markers and no accepted treatment. As such, it is best considered a syndrome with a pathophysiologic basis explained in terms of a "multiple hits hypothesis." Some propose that steatosis precedes inflammation, while others propose the opposite, i.e., that inflammation precedes and besets steatosis.[26,27] Simple hepatic steatosis is benign and nonprogressive, unlike NAFLD/NASH. The observation that anti-TNF-α treatment and metformin (an antidiabetic drug that inhibits hepatic TNF-α expression) improve steatosis in the ob/ob (Leptin-deficient) mouse model used to study NASH supports a link between NAFLD/NASH and inflammatory mediators.[28,29] Macrophages and other cell types within adipose tissue and liver initiate and perpetuate an environment provoking hepatic lipid accumulation.[30-34] As such, hepatic steatosis in NAFLD/NASH is considered a "bystander phenomenon" in which hepatic fat accumulation, inflammation and progressive insidious liver failure (in NASH) coordinate with escalating insulin resistance.

Nutritional & Endotoxin Initiators: Both the gastrointestinal tract and VAT have direct splanchnic circulatory access to the liver (portal circulation). Consequently, diverse processes in these tissues associated with ingestion of "toxic" lipids (FFA, transfats, saturated FA) and other nutrients, as well as inflammatory mediators, signaling molecules and hormones made locally in the alimentary tract, can detrimentally influence hepatic metabolism. In man, ingestion of a high-fat or high-carbohydrate diet for only three days increases circulating lipopolysaccharide (LPS/endotoxin) that induces markers of systemic inflammation. Increased circulating LPS is linked to worsening obesity and has been postulated to reflect enteric translocation and perhaps small intestinal bowel overgrowth (SIBO) associated with obesity (Figure 4).[32] Using experimental NASH (mouse) models, four weeks of endotoxemia combined with a high-fat diet increases liver and adipose TG and induces hepatic insulin resistance.[31,32]

Since these effects are preventable by antimicrobial administration causally implicates enteric microbial

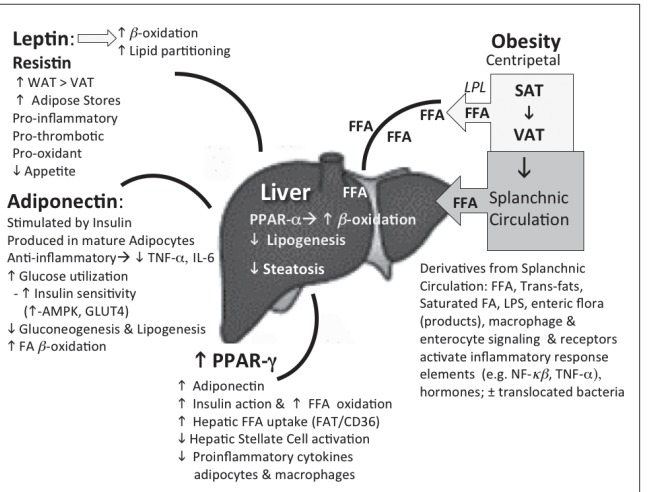


Figure 4

flora and products.[31] Consistent with this hypothesis is that increased gut permeability in humans with NAFLD correlates with severity of hepatic steatosis (but not degree of NASH).[32] It is widely accepted that inflammatory bowel disease and enteric translocation produce secondary hepatic lesions (so-called "reactive hepatitis"), where innate enteric immune responses are implicated (e.g., toll-like receptors expressed in gut epithelium). These, in turn, activate inflammatory response mediators (e.g., activation of NF-κβ) inducing pro-inflammatory cytokines (in macrophages and enteric epithelium) dispersed to the liver in the splanchnic circulation. Because translocation through the splanchnic circulation can result in both hepatic lesions and systemic insulin resistance (animal models), endotoxin is a proposed "second hit" NAFLD/NASH mediator.

Nomenclature & Definitions (Table 2):
Lipases, Enzymes, Nuclear Receptors & Transporters Involved with NAFLD/NASH Acetyl-CoA Carboxylase (ACC):
Catalyzes the carboxylation of acetyl-CoA forming malonyl-CoA, a key molecule controlling intracellular FA metabolism. ACC regulates FA synthesis and indirectly controls FA oxidation through malonyl CoA formation. Reduced ACC promotes accumulation of intermediate

Table 2: Noteworthy adipokines, nuclear receptors or effectors relevant to metabolic syndrome, non-alcoholic fatty liver disease and non-alcoholic steatonecrosis. Adapted but modifed from references 11, 12

Adipokine	Activator	Inhibitor	Action
Adiponectin	Weight loss	TNF-α, IL-6, resistin, insulin, glucocorticoids	↑ mitochondrial β-oxidation via ↑ AMPK (activated protein kinase); ↓ lipogenesis by ↓SREBP-1c; hepatic anti-inflammatory/anti-fibrotic by antagonizing TNF-α and ↑ Stellate cell apoptosis, ↑ FFA oxidation by ↑ PARR-α; ↑ insulin sensitivity **(↓ hepatic lipid)**
Leptin	Overfeeding, obesity, glucocorticoids, insulin	Fasting, sustained, exercise, cold exposure, weight loss	central anorexigenic; insulin sensitizer; ↑ mitochondrial FFA oxidation by ↓ Malonyl-CoA synthesis, ↑ AMPK activation of ATP-producing catabolic pathways (e.g. β-oxidation, glycolysis); ↓ ATP consuming anabolic pathways; profibrogenic ↑ hepatic stellate cell activation; **(↑ hepatic lipid)**
TNF-α (tumor necrosis factor-α)		Certain drugs/ antibodies, antioxidants	↑ glucose resistance by ↓ GLUT4 & ↑ LPL in adipocytes & hepatocytes;↑ hepatic inflammation (stress pathways:JNK-1, NF-κβ, IL-6); ↓ adiponectin/ adiponectin receptor; ↑ hepatic β-oxidation, ↓ VLDL formation,↑ lipogenesis **(↑ hepatic lipid)**
Resistin	TNF-α, IL-6, IL-1, LPS		↑ glucose resistance by ↑ AMPk activation & ↓ GLUT-4 in adipocytes; ↑ nuclear translocation NF-κβ; ↑ macrophage TNF-α; ↑ resistin correlates with NAFLD histologic severity and ↑ hepatic glucose metabolism **(↑ hepatic lipid)**
IL-6 (Interleukin- 6)	Saturated fatty acids		↓ adiponectin secretion; ↓ lipoprotein lipase (LPL); ↑ SOCS-3 secretion; pro-inflammatory in liver by Kupffer cell activation **(↑ hepatic lipid)**
SOCS-3 (suppressor of cytokine signalling-3)	TNF-a, IL-6 , leptin	Adiponectin	↑ *de novo* lipogenesis via ↑ hepatic SREBP-1c; ↑ insulin resistance (phosphorylation of insulin receptor substrate) **(↑ hepatic lipid)**
Nuclear Receptor/Effector	**Activator**	**Inhibitor**	**Action**
PPAR-α (peroxisome proliferator-activated receptor) in hepatocytes, muscle, heart	PUFA, fibrates, eicosa-noids, adiponectin		↑ hepatocyte FFA uptake by fatty acid translocase (FAT/CD36); ↑ mitochondrial & peroxisomal β-oxidation; ↓ NF-κβ signaling pathway **(↑ hepatic lipid)**
PPAR-γ (peroxisome proliferator-activated receptor) in adipocytes, hepatocytes, stellate cells, pancreatic β-cells macrophages, endothelium	Thiazolidinediones	MeTS, inflammatory adipocytokines	↑ insulin action; ↑ FFA oxidation; ↑ adiponectin secretion, ↑ hepatocyte FFA uptake by fatty acid translocase (FAT/CD36); ↓ macrophage activation; ↓ hepatic stellate cell activation, ↓ secretion of pro-inflammatory cytokines by adipocytes & macrophages **(↓ hepatic lipid)**
SREBP-1c (sterol regulatory element binding protein) in adipocytes & hepatocytes	Insulin, endocannabi-noids, SOCS-3 protein, homocysteine; ER stress, saturated-FA, trans-FA	Leptin, glucagon, PUFA, AMPK	↑ transcription of lipogenic enzymes: acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), stearoyl-CoA desaturase-1 **(↑ hepatic lipid)**
Oxidative & ER "Stress"	**Activator**	**Inhibitor**	**Action**
Oxidative Stress	Mitochondrial dysfunction, Toxic FFA, inflammatory adipocytokines	Antioxidants, upregulation of adiponectin	Activates redox sensitive pathways: JNK, NF–κβ, protein kinase C;↓ VLDL formation/secretion; ↑ insulin resistance **(↑ hepatic lipid)**
Endoplasmic Reticulum Stress	Unfolded protein response, Oxidative injury		↑ ROS formation, JNK activation; ↑ lipogenesis via SREBP-1c;↑ insulin resistance **(↑ hepatic lipid)**
Endotoxin	Enteric translocation, microbiota, certain foods		Toll -like-receptor-4 (TLR-4) dependent ↑ NF-κβ; may ↑ TNF-α, IL-6 **(↑ hepatic lipid)**

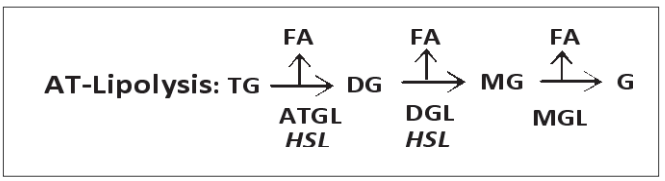
saturated FA and diacylglycerol that can trigger hepatocyte apoptosis and insulin resistance. ACC transcription is enhanced by SREBP-1c (see below) and ChREBP (see below) and downregulated by AMPK (adenosine monophosphate-activated protein kinase system) (see below).

Adenosine Monophosphate-Activated Protein Kinase (AMPK): Activation directly and indirectly modulates several key metabolic pathways influencing obesity and its co-morbidities (including NAFLD/NASH). ACC isoforms (see above) are direct substrates of AMPK, where phosphorylation leads to ACC inactivation. Increased AMPK activation therefore decreases lipogenesis and increases FA oxidation. Hormone-sensitive lipase (HSL) also is an AMPK target. AMPK suppresses lipogenic genes by inhibiting expression or activation of a key transcription factor, sterol regulatory element binding protein 1c (SREBP1c). In skeletal muscle, AMPK stimulates glucose uptake via the insulin-responsive transporter (GLUT4) system. Hepatic AMPK activation attenuates glucose production (gluconeogenesis and glycogenolysis), although the exact mechanisms are not yet clear.

Fatty Acid Synthase (FAS): Catalyzes *de novo* synthesis of long-chain fatty acids (LCFA) in adipose and other tissues, including liver. FAS is a complex enzyme "system" rather than a single molecule.

Lipolysis Enzymes

Hormone-sensitive lipase (HSL) gene = LIPE): Is a cytosolic enzyme that in addition to three other lipases (triglycerol hydrolase [liver expression], adipose triglyceride lipase [ATGL], and monoglycerol lipase) orchestrates adipose TG hydrolysis. Predominantly expressed in adipose, HSL plays a crucial role regulating lipolysis (rate limiting for diglyceride hydrolysis) and has broad substrate specificity. (See diagram: AT=adipose tissue,



TG=triacylglycerol, FA=fatty acid, DG=diacylglycerol, DGL=diacylglycerol lipase, MG=monoacylglycerol, MGL=monoacylglyceride lipase, and G=glycerol)

Relative maximal hydrolysis rates are in the range of 1:10:1:4:2 for triglycerides: diglycerides:monoglycerides: cholesterol esters: retinyl esters.[35,36] Thus, TG are the worst substrate, and diglycerides are the best. HSL-mediated lipolysis is activated through beta-adrenergic signaling pathways, increased by heparin and catecholamines and blocked by insulin (insulin's anti-lipolytic activity). β-adrenergic stimulation involves increased cellular cAMP followed by activation of protein kinase A (PKA). PKA phosphorylates cytosolic HSL and the lipid droplet-association protein, perilipin A, leading to translocation of HSL to the lipid droplet interface where HSL initiates hydrolysis.[35,37,38] Phosphorylation of both proteins is requisite for HSL-mediated lipolysis (increases activity 100-fold).[35] HSL is downregulated during acute fasting and increases only after prolonged food deprivation (three to five days).[35]

Adipose triglyceride lipase (ATGL; gene=PNPLA 2 for patatin-like phospholipase domain containing protein-2; alternative names are desnutrin, and phospholipase A2ζ and transport-secretion protein): Is highly expressed in WAT and performs the initial step in TG hydrolysis.[35-38] ATGL is responsible for the basal lipolytic rate independent of HSL with its activation independent of PKA-mediated phosphorylation. ATGL is located on the lipid droplet interface in the basal- and hormone-stimulated state and thus does not require translocation to initiate hydrolysis. Rather, ATGL activity is regulated by an activator protein (CG1-58: comparative gene identification 58). ATGL mRNA expression is markedly influenced by nutritional status, increasing during fasting and decreasing during refeeding.[35,36] Expression is reduced by insulin (rodent models). The role of ATGL in MeTS, NAFLD and NASH is relatively uninvestigated. However, ATGL is a major hepatic TG lipase playing an integral role in the FFA partitioning and signaling that controls energy metabolism (e.g., protects the liver from steatosis by increasing hepatic TG hydrolysis and β-oxidation and increasing PPAR-α and its target gene expression).[39] Despite extensive knowledge regarding specific lipase activity, β-adrenergic stimulation initiates lipolysis independent of ATGL or HSL mRNA expression, suggesting post-transcriptional regulation.[35]

Triglycerol hydrolase (TGH, two isoforms): Is highly expressed in liver and is more efficient in hydrolyzing substrates esterified with short-chain fatty acids.

Monoglycerol lipase (MGL): Is rate limiting for monoacylglycerol hydrolysis, releasing the third FA from the glycerol backbone (yielding glycerol and FFA).

Sirtuin 1 (Sirt-1): Is a nicotinamide adenine dinucleotide (NAD+)-dependent deacetylase that enhances hepatic gluconeogenesis and fatty-acid oxidation, reduces lipogenesis, and has an anti-inflammatory effect likely through suppression of NF-κβ signalling.[40] It also promotes adipose

adiponectin expression known to activate Sirt-1. It also has been associated with mitochondrial biogenesis thereby sustaining fatty-acid oxidation. During prolonged starvation, SirT-1 downregulates expression of key gluconeogenic genes. Severe Sirt-1 repression recently has been shown in humans with NAFLD, where it is thought to promote the MeTS phenotype.[41] This could reflect impaired mitochondrial biogenesis and fatty-acid oxidation that could promote severe hepatic steatosis in morbidly obese individuals. Furthermore, Sirt-1 is implicated in NAFLD/NASH as a result of work with the Sirt-1 hepatocyte specific deletion mouse model that develops marked hepatic steatosis, ER stress and liver inflammation.[42]

Adiponutrin 3 (Patatin-like Phospholipase 3 [PNPLA3] gene): Is a transmembrane protein with phospholipase, TG lipase and acylglycerol transacylase activity (transfers fatty acids to mono- and di-acylglycerol) that is expressed in liver and adipocytes. Adiponutrin is induced during adipocyte differentiation and in response to fasting and PPAR-γ activation and is downregulated by insulin and TNF-α. Genomewide-association population-based studies in humans and small cohort studies have identified a single nucleotide polymorphism (rs738409, G allele, encoding isoleucine substitution for methionine [1148M]), causing a missense mutation that associates with hepatic steatosis (different ethnic groups, independent of obesity and diabetes status).[12,43] The polymorphism is most common in humans of Southern European ancestry. A recent study (Dallas, Texas) showed that this allele mutation may account for virtually all ethnic differences in NAFLD frequency (~40% Hispanics, ~30% Europeans, ~20% African Americans). The mutation correlated with increased ALT activity and is linked with higher rates of NASH and fibrosis with NAFLD and alcoholic liver disease.[43,44] Yet the actual mechanism of PNPLA3 in NAFLD/NASH remains unclear at present based on deductions made in knock-out mouse models.

Inflammatory Mediators: (Figure 5)
Monocyte-chemoattractant Protein-1 (MCP-1): Activates macrophage recruitment and stimulates production and release of TNF-α and other inflammatory mediators. It also stimulates lipogenesis via SREBP-1c.[11]

Peroxisome Proliferator Receptor-alpha (PPAR-α): Is a transcription factor shown to be downregulated in NASH.[11] PPAR-α is the master fatty-acid oxidation-governing transcription factor PPAR-α that increases FFA uptake into hepatocytes and myocytes through FAT/CD[36] (see below) expression; increases mitochondrial and peroxisomal β-oxidation; inhibits the NF-κβ pathway (imparting

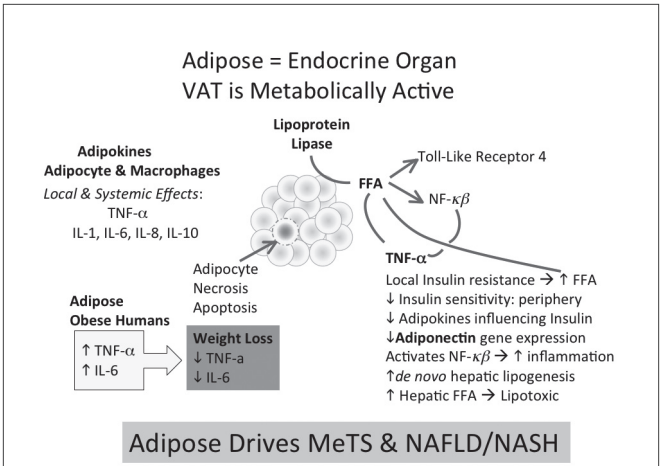


Figure 5

an anti-inflammatory effect); and enhances insulin sensitivity. PPAR-γ is activated by PUFA (polyunsaturated fatty acids), fibrates (drugs), eicosanoids and adiponectin.

Peroxisome Proliferator Receptor-γ (PPAR-γ): Is a transcription factor that enhances insulin action, FFA oxidation, adiponectin secretion, hepatocyte FFA uptake (via FAT/CD36) (see below), inhibiting macrophage and hepatic stellate cell (HSC) activation and secretion of pro-inflammatory cytokines by adipocytes and macrophages. Downregulation of PPAR-γ is associated with MeTS and NAFLD/NASH. PPAR-γ can be activated by the thiazolidinedione agents (e.g., pioglitazone) resulting in a decrease in hepatocellular lipid stores and insulin resistance in adipose tissue in MeTS and NAFLD/NASH.

Steroid Response Element Binding Peptide-1c (SREBP-1c): Enhances lipogenic enzyme transcription (e.g., ACC, acylCoA:diacylglycerol acyltransferase 1, fatty-acid synthase [FAS], stearoyl Co-A desaturase-1 [SCD-1]). SREBP-1C is activated by insulin (increased nuclear expression), endoplasmic reticulum stress, saturated FA and trans-FA, and is inhibited by leptin, glucagon, PUFA and AMPK.

Carbohydrate Response Element Binding Protein (ChREBP): Enhances transcription of the lipogenic enzymes ACC and, fatty-acid synthase (FAS). CHREBP is stimulated by glucose and, accordingly, is increased by insulin resistance.

Fatty Acid Transport Into Cells
Fatty Acid Transporters: FFA uptake occurs by both passive and facilitated mechanisms. Fatty-acid membrane transporters facilitate long-chain FA uptake (c12-C20). Three relevant saturable transmembrane carrier families are defined: fatty-acid transport proteins (FATPs), 6 iso-

forms with different tissue distribution; fatty-acid translocase, cluster differentiation protein-36 (FAT/CD36); and fatty-acid binding proteins (FABPs), 9 isoforms. Overexpression of FA transporter proteins promotes steatosis, insulin resistance and dyslipidemia. Induction of FAT/CD36 enhances facilitated hepatic FFA uptake in fat and muscle and is implicated mechanistically in NAFLD/NASH; as indicated previously, FAT/CD36 induction occurs secondary to PPAR-α and PPAR-γ expression.

Glucose Transport Into Cells

Glucose Transporters: Insulin facilitates glucose entry into muscle, fat and some other tissues by increasing glucose transporters at the cell surface. The major insulin sensitive transporter is GLUT-4 orchestrating 90% of glucose uptake by 10- to 40-fold in insulin-sensitive tissue (i.e., muscle and fat).[45] The GLUT-1 transporter is expressed in most tissues (i.e., brain, RBC, blood-brain barrier, many other tissues) and accounts for a relatively small amount of total glucose uptake in skeletal muscle and adipose. It is considered insulin insensitive and imparts transport effects at basal level expression.[46] Reduced GLUT-4 expression is an early change in human insulin resistance. [46]

Adipokines (Table 2, Figures 4 and 6)

Concepts central to the understanding of NAFLD/NASH are: 1) that adipose tissue is a highly reactive endocrine and immune organ, and 2) that obesity is a systemic, low-grade inflammatory disorder in which adipose and its hormones play a central role.[10-12,34] A growing body of work has characterized diverse adipocytokines, biologically active substances produced locally in adipose acting in autocrine/paracrine or endocrine fashion. A brief profile of adipocytokines relavent to NAFLD/NASH is provided below.

Leptin: Expressed by adipocytes, more leptin is derived from white adipose tissue (WAT) as compared to VAT, with expression increasing with expanding TG stores. Considered pro-inflammatory, pro-thrombotic and pro-oxidant, leptin normally contributes to appetite suppression through central neural sensors. Leptin concentrations correlate with adipose mass with appropriate physiologic responses enhancing FA β-oxidation (inhibit malonyl-CoA synthesis, stimulating AMPK-activating, ATP-producing catabolic pathways [e.g., β-oxidation and glycolysis]), inhibiting ATP-consuming anabolic pathways and directing appropriate lipid partitioning to subcutaneous fat (SAT).[47] However, because leptin chronically increases in obesity, acquired leptin resistance impairs its normal effects. Like TNF-α and IL-6 (described below), leptin promotes hepatic fibrogenesis through activation of HSC by directly binding with the HSC receptor or by stimulating hepatic Kupffer cells to produce transforming growth factor-β (TGF-β). TGF-β activates HCS to its fibrogenic phenotype.[34] Yet, leptin resistance may play its most influential role in MeTS and NAFLD/NASH through its influence on appetite and energy utilization.

Adiponectin: Produced by mature adipocytes, adiponectin is anti-inflammatory (mediated in part by inhibition of macrophage TNF-α production), improves glucose utilization by increasing insulin sensitivity, opposing insulin resistance, and inhibiting hepatic gluconeogenesis, and stimulates FA β-oxidation (inactivates ACC). Adiponectin enhances insulin sensitivity though AMPK, opposing lipogenesis by ACC phosphorylation. It increases glucose uptake via the GLUT4 glucose transporter and increases glycolysis (via phosphorylation of phosphofructokinase). While adiponectin production is stimulated by insulin, insulin resistance downregulates the adiponectin receptor expression resulting in "adiponectin resistance." Because hypertrophied adipocytes and adipocytes expanded with TG stores develop insulin resistance, secreting less adiponectin and adiponectin concentrations inversely correlate with body fat mass.[48] Thus, in humans, low adiponectin levels associate with obesity, type-2 diabetes mellitus, NASH, essential hypertension and coronary artery disease.[49] Upon resolution of obesity, adiponectin concentrations normalize and adiponectin resistance disappears.[50] Adiponectin does not appear to influence glucose uptake, glycogen synthesis or glycogenolysis in liver in humans or rodents.

As an anti-inflammatory adipocytokine, adiponectin protects against development of steatohepatitis, switching metabolic utilization of fat by inhibiting lipogenesis (downregulating SREBP-1c) and activating fatty-acid oxidation (via AMPK and PPAR-α).[11] In addition to antagonizing the action of TNF-α, it promotes HSC apoptosis, thereby limiting fibroplasia.[12] Obesity, MeTS and NASH associate with hypoadiponectinemia, a state permissive to hepatic inflammation and compromised lipid partitioning. The net effect is ectopic fat accumulation in VAT and hepatocytes and development of an inflammatory milieu in VAT and liver.

Several lines of evidence support a pathophysiologic role of hypoadenonectinemia in NAFLD/NASH. Adiponectin signalling occurs via two receptors: receptor-1 activates AMPK and Sirt-1.[26] Adiponectin knock-out mice develop fibrosing steatohepatitis when fed a high-fat diet and the knock-out Sirt-1-mouse develops hepatic steatosis, endoplasmic reticulum stress and hepatic in-

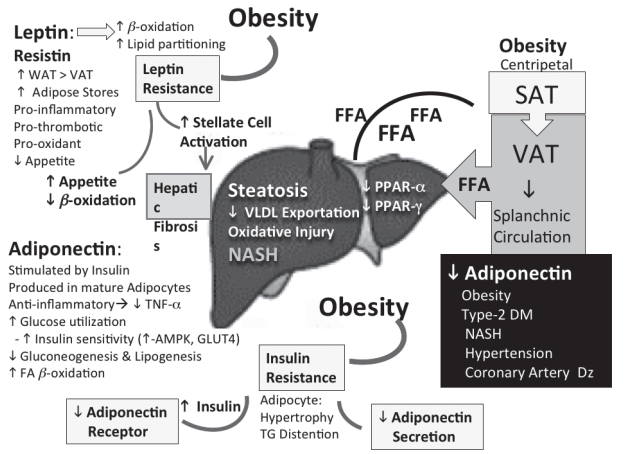


Figure 6

flammation.[33,51-53] Furthermore, overexpression of adiponectin in the leptin-deficient mouse (ob-/ob-) resolves its diabetic phenotype, insulin resistance and hepatic lipidosis, despite retention of systemic obesity.[51]

Angiotensinogen and the Renin-Angiotensin System (RAS): Both play a role in normal adipocyte differentiation and metabolism.[53] WAT is a major source of angiotensinogen, second only to the liver.[54] Fat also contains RAS-converting enzymes. In humans, increased production of angiotensinogen in obesity (WAT) contributes to obesity-related cardiovascular and renal disease. Greatest effects on the RAS derive from increased VAT, characteristic of MeTS, with increased angiotensinogen spilling into the systemic circulation. Enhanced RAS activity in adipose also contributes to local inflammation. Increased angiotensin II promotes MeTS and insulin resistance by inhibiting insulin-stimulated GLUT4 translocation and increasing inflammatory cytokine signalling.[55] Angiotensin II stimulates adipocyte leptin production, whereas suppression of the RAS increases adiponectin.

Resistin: This has variable origin in different species, including macrophages, adipocytes and leukocytes, and is known to increase in obesity. Effects are similar to leptin, with resistin contributing to insulin resistance and metabolic derangements associated with type 2 diabetes.[11,12] It also is pro-inflammatory associated with upregulation of vascular adhesion molecules and increased macrophage production of pro-inflammatory cytokines.

Visfatin: This recently discovered adipokine exerts insulin-mimicking effects and activates the insulin receptor in a manner distinct from insulin.[56] Some evidence supports possible coregulation with IL-6 (inflammatory cytokine), but its role in NAFLD/NASH is not fully established.

Inflammatory Adipokines/Cytokines (Figure 6, Table 2) Considering the numerous interacting or shared molecular mechanisms involved with MeTS and NAFLD/NASH, it is widely accepted that adipose tissue generates mediators (adipocytokines and cytokines) with both systemic and hepatic effects. Rapid adipocyte hypertrophy without appropriate cell proliferation causes ectopic lipid deposition in liver and VAT along with local insulin resistance. Key adipocyte proteins governing fat uptake into adipose and muscle, such as FAT/CD36, phospholipases, including members of the adiponutrin family, and the HSL family of enzymes (controllers of adipose lipolysis), have each been implicated.[12] Chemikines (e.g., MCP-1) released from hypertrophied adipocytes locally recruit macrophages initiating and sustaining a vicious cycle that drives inflammation and suppresses anti-inflammatory adipokines. One theory maintains that macrophages recruited into an expanding adipose mass release chemoattractant chemokines in response to increased adipocyte apoptosis and necrosis.[57] Increased expression/secretion of pro-inflammatory adipokines (e.g., TNF-α, IL-6) also provokes insulin resistance. Coupled with obesity-related down-regulation of anti-inflammatory and anti-lipotoxic adipokines (e.g., adiponectin), the stage is set for self-perpetuating cytokine production/release and insulin resistance.

Notable mediators derived from macrophages within adipose tissue include TNF-α, interferon-γ, IL-1, IL-6, IL-8, IL-10, MCP-1, and complement proteins.[50,54] While MCP-1 can activate inflammatory pathways, it also directly promotes hepatocyte TG accumulation.[11] In addition to these mechanisms, increased circulating FFA concentrations (characteristic of obesity) activate innate immune responses via toll-like receptor-4 and by enhancing NF-κβ signaling.[59,61]

TNF-α & IL-6: The most studied chemokine in relation to MeTS and NASH, TNF-α induces local insulin resistance increasing FFA release into the systemic circulation and diminishing insulin sensitivity in peripheral tissues.[60-66] TNF-α also alters secretion of other adipokines, influencing glucose metabolism (i.e., reduces gene expression of adiponectin).[60,62,63] IL-6 and TNF-α expression are markedly increased in fat cells from obese humans with insulin resistance, with weight loss significantly abrogating their expression.[62,63] One common link among increased cytokine expression, MeTS and NAFLD/NASH is through stimulation of insulin production. An experimental mouse model has demonstrated an increase in IL-6 in response to a high-fat diet that subsequently leads to hyperinsulinemia, hepatic steatosis and hepatic insulin resistance.[67] Notable is that the high-fat diet behaves as an "inflammatory diet" driving liver pathol-

ogy and cytokine expression, resembling the apparent link among diet, hyperlipidemia, atherosclerosis and MeTS in humans.[26] Strikingly, weight loss abrogates adipose IL-6 expression and other induced inflammatory mediators and the inflammatory phenotype.

Compared to patients with simple steatosis, those with NASH have enhanced hepatic TNF-α expression (peripheral circulation, adipose tissue).[66] In experimental models of NAFLD/NASH and in affected humans, TNF-α critically promotes syndrome development and progression with VAT macrophages serving as the primary cytokine source.[60] TNF-α activates NF-κβ initiating release of pro-inflammatory cytokines and also may initiate *de novo* hepatocellular lipogenesis.[66,67] Interruption of TNF-α signalling, either pharmacologically or by genetic manipulation, reduces hepatic lipid accumulation in NAFLD/NASH models.[68,69] However, the cause or effect relation is unclear as experimentally increased hepatic FFAs induces TNF-α production and hepatic lipotoxicity.[67]

FAS & PPAR-γ Receptors: Two cytokine receptors associated with adipose and MeTS are the FAS death receptor and the PPAR-γ receptor. FAS-deleted mice are protected from adipose inflammation, hepatic steatosis and hepatic insulin resistance induced by a high-fat diet.[70] Thus, FAS receptor is mechanistically implicated as a signaling pathway in MeTS. The PPAR-γ receptor plays an important role in adipogenesis, atherosclerosis, inflammation and glucose metabolism. Deletion of the adipose-specific PPAR-γ receptor (in mice) reduces weight gain despite hyperphagia and diminishes serum leptin, adiponectin and insulin resistance.[71,72] Thus, evidence suggests that upregulation of PPAR-γ may be beneficial.

Toxic Lipids & Endoplasmic Reticulum (ER) Stress (Figure 7): Chronic imbalance between energy intake and utilization in obesity exposes cells to accumulated "toxic lipids" that activate cellular stress responses. Thus, a component of pathophysiology of MeTS having relevance to NASH is generation of ER stress, known to stimulate lipogenesis through SREBP-1c. ER stress involves the accumulation of "unfolded" or "misfolded" proteins within the organelle and triggers adaptive responses aimed at stress resolution ("unfolded" protein response or [UPR]).[73] The UPR is mediated by three stress-sensing pathways (pancreatic ER kinase [PERK], inositol requiring enzyme [IRE-1] and activating transcription factor 6 [ATF-6]) that intersect and interact with other signals promoting obesity and its related insulin resistance and inflammation.[73,74] While increased delivery and uptake of FFA may induce ER stress, other adipose-derived unsaturated fatty acids (lipokines, such as palmitoleate) may inhibit or diminish

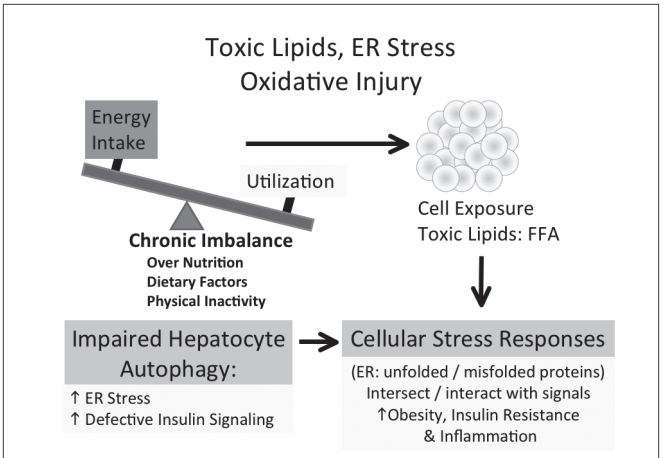


Figure 7

ER stress.[75,76] Furthermore, storage of fat as TG may provide a protective effect against FFA exposure.

Oxidative Injury: Immunohistochemical techniques marking the presence of oxidation products (e.g., 4-HNE, 8-hydroxy-deoxyguanosine and nitrotyrosine as a marker of peroxynitrite formation) in liver tissue from humans with NAFLD show more intense reactivity in NASH biopsies compared to simple steatosis.[77-80] Increased liver and serum lipid peroxidation products and reduced plasma antioxidant status also have been shown in NAFLD.[23,80] Expression of antioxidant enzymes also is lower in liver tissue from patients with NASH compared to other liver disorders, further substantiating a likely role of oxidative injury.[81]

Impaired Hepatic Autophagy (Figure 7): Autophagy (self-autodigestion) may additionally influence hepatic lipid metabolism leading to steatosis. This phylogenetically conserved response to cellular starvation regulates lipid metabolism by inhibiting increases in cell TG stores.[82,83] Impaired (reduced rate of) autophagy is thought to increase ER stress and to promote defective insulin signaling (resistance).

Subcutaneous & Visceral Adipocytes-Role in NAFLD/NASH: VAT and SAT differ in their metabolic functions and secretory products (Table 3); these differences explain why increased VAT, rather than SAT, correlates best with insulin resistance and development of NAFLD/NASH.[84,85] Overflow of VAT adipokines and FFA to the liver are causal to NAFLD/NASH and can be abrogated by weight loss and treatment with insulin sensitizers. However, increased SAT also contributes to physiologic dysregulations promoting adipose lipolysis and ectopic fat partitioning (to the liver) in NAFLD/NASH. In health, SAT functions as a rapidly expandable reservoir of small

Table 3: Relative expression of adipocytokines in visceral and subcutaneous adipose tissue. Adapted from reference 84

Adipocytokine	Comparative Expression
Adiponectin	VAT < SCAT
Acylation stimulating protein	VAT < SCAT
Leptin	VAT < SCAT
TNF-α	VAT = SCAT
Angiotensinogen	VAT > SCAT
IL-6	VAT > SCAT
IL-8	VAT > SCAT
PPAR-γ	VAT > SCAT
Resistin	VAT > SCAT

VAT = visceral adipose tissue;
SCAT = subcutaneous adipose tissue

insulin-sensitive adipocytes that absorb excess FFA and TG during the postprandial interval. Normal insulin response enables proliferation and maturation of "pre-adipocytes" in SAT expanding this "buffering" capacity. Attenuation of this response deviates FFA to VAT and onward to hepatocytes. Compared to SAT, VAT adipocytes are large, having an expanded storage capacity, but are less responsive to insulin and foster a chronically enhanced lipolytic milieu. VAT adipocytes release more pro-inflammatory cytokines and less leptin (Table 3) compared to SAT. Adiponectin and PARR-γ promote polarization of adipose macrophages (source of inflammatory cytokines) toward an anti-inflammatory phenotype.[86] VAT macrophages may transit directly to the liver through the portal vein, where they directly influence (impair) hepatic insulin signalling and release pro-inflammatory mediators (e.g., TNF-α, IL-6). Induced hyperinsulinemia and hyperglycemia exert multiple effects on hepatic metabolism and growth including increased nuclear expression of SREBP1c (steroid response binding protein, related to high insulin) and induction of ChREBP (carbohydrate response binding protein, related to high glucose) that stimulate hepatic lipogenesis. Insulin resistance, suppressed FA β-oxidation, impaired VLDL formation and enhanced FAT/CD36 expression (increases hepatocyte FFA uptake) collaboratively promote hepatic lipid accumulation. Different animal models of NAFLD/NASH have elucidated how diverse alterations in adaptive pathways initiate or drive metabolic dysregulations favoring hepatic lipid accumulation, chronic inflammation and fibrogenesis. These models have elucidated how oxidative stress and mitochondrial injury secondary to hepatic lipid (FFA) deposition and macrophage activation augment FA and TG accumulation through impaired FA oxidation.[88,89]

Yet, there is increasing evidence that hepatic fat accumulation is not always pathologic but rather represents an appropriate physiologic response to increased energy intake and mobilization. In this respect, TG synthesis is an adaptive, beneficial response detoxifying potentially toxic fat metabolites (e.g., FFA).[90] FFA and cholesterol, especially when accumulated within mitochondria, represent injurious lipids and promote TNF-α mediated liver injury along with and formation of reactive oxygen species (ROS).[88,89] FFA and cholesterol are currently believed to act as early inflammatory hits in NAFLD/NASH, preceding severe TG accumulation.

Over-Nutrition/Obesity/NAFLD/NASH (Figure 5): The other component essential in the pathophysiology of NAFLD/NASH is overnutrition. This complex biologic phenomenon reflects multiple abnormalities involving central appetite regulation, food/energy intake, energy expenditure and metabolic regulation beyond the intent of the current discussion.

Dietary Factors: In humans, many environmental factors influence diet composition (economic, cultural, behaviors) and thus play a facilitatory role sustaining the imbalance between energy intake and utilization, driving the pandemic of overnutrition. Yet, energy excess alone does not discriminate among development of NASH, simple steatosis or normal liver histology. Inconsistent associations exist between NASH and the type of fat ingested (saturated, nonsaturated, trans fats, PUFA) and the level of carbohydrates consumed. However, evidence does suggest that trans-fat ingestion may cause greater hepatic insulin resistance and necroinflammation, IL-1 and TNF-α gene expression. Studies in humans also suggest a link between dietary cholesterol ingestion and cirrhosis, irrespective of cause, and that a low intake of antioxidant vitamins A, C, and E associate with risk for NASH.

Physical Activity-Obesity/NAFLD/NASH: Increasing aerobic activity on a regular basis improves metabolic indices and adipokine profiles strongly associated with NAFLD/NASH. Lifestyle changes incorporating exercise and dietary modifications indeed have altered histologic features improving NASH lesions in individual patients

Genetic Mechanisms – Obesity: While ~100 genes are speculated to associate with obesity, obesity in humans segregates as a complex trait. As such, it is hypothesized that a combination of as many as 30 genes may be involved in the obese human phenotype.[11,12] Fatty-liver disease often has a familial association and is more common in certain ethnic groups. Increased hepatic TG content (detected using MRS/I) varies from ~20% in African American and European women to ~30% in European men and 40% in Hispanics.[11,12] Family clustering, adoption and twin studies have calculated heritability of

obesity to be between 0.6 to 0.7. Populations with historical hunter-gatherer lifestyle behaviors who have recently adapted to Western cuisine (fast foods) have exceptionally high rates of obesity and MeTS. Low socioeconomic status also increases human risk for MeTS and NAFLD/NASH.

Some genes contributing to an obese phenotype may act in the hypothalamus or brain stem sensing fat stores and signaling appetite. Rare genetic variations in the HSL family (controls adipocyte lipolysis) have been linked with obesity, glucose intolerance and dyslipidemia. Recently, increased risk for NASH was associated with the Patatin-like phosphatase family (nine genes with five designates as the adiponutrin family [PNPLA1-5]).[11] These proteins express in WAT and liver and functionally complement HSL. A PNPLA-3 polymorphism encoding adiponutrin-3 associates with hepatic steatosis (studies with different ethnic groups, independent of obesity and diabetes status) and also with severity of fibrosis in NASH.[91]

Source of Hepatic Lipid in NAFLD & NASH: Lipid metabolism in the liver involves three categories: 1) *accumulaiton*: by *de novo* synthesis or active uptake of FFAs; 2) *utilization*: by β-oxidation and/or *de novo* synthesis of TG; and 3) *storage or exportation*: by incorporation of FFAs into TG for storage or formation of very low-density lipoproteins (VLDL) released into the systemic circulation. Several plasma membrane protein transporters govern FA uptake into cells. The main translocation process involves members of the FA transporter protein (FATP) family that facilitate uptake of long-chain FA (C12–C20). Highly expressed in hepatocyte and adipocyte membranes, FATP maintains high-level FA uptake for both metabolism and storage. Six members of the FATP family (FATP1–6) are known with organ specific isoform distributions.[6 92,93] FATP-5 facilitates hepatic FA uptake, expression correlating with serum FFAs, hepatocyte apoptosis, and fibrosis in NAFLD. Upregulation of FATP-5 occurs simultaneously with FAT/CD36 expression, with FAT/CD36 facilitating active hepatic fatty acid uptake induced by insulin.[94] Thus, the effect of increased hepatic FFA delivery is exaggerated by enhanced FFA uptake secondary to increased transporter expression. Dynamic adjustment of FAT/CD36 expression, shown using several knock-out NASH models, reflects dietary FA, hormonal changes (associated with MeTS), and expression of nuclear transcription factors (e.g., liver X receptor, PPAR-γ) influenced by macrophage cytokines and adipokines.

Both increased insulin and glucose drive lipogenesis by the respective transcription factors SREBP1c and ChREBP.[11] While rodent models confirmed the role of hyperinsulinemia in the early stages of insulin resistance and susceptibility to hepatic steatosis, studies using the SREBP1c overexpressing mouse model show that hepatic lipogenesis leads to steatosis but not necessarily to NASH. Furthermore, excessive fat ingestion and subsequent VAT lipolysis increase serum FFAs and development of "simple" hepatic steatosis but not necessarily NASH. So, while studies in humans with NAFLD/NASH implicate enhanced *de novo* lipogenesis and increased delivery of FFA (and other lipids) from the diet, a more significant contribution of hepatic fat derives from peripheral fat stores and mechanisms increasing hepatocellular lipid uptake or impairing hepatic catabolism or export of FA.

In healthy humans, VAT contributes 82% of the FFA pool during fasting and 62% after feeding.[95] Humans with NASH derive ~60% of their hepatic TG from adipose FFA (VAT) compared with only ~25% from *de novo* lipogenesis. While excessive adipose stores increase risk for NAFLD/NASH, it is the increased hepatic FFA concentrations rather than TG that are implicated in the pathogenesis of NASH and that distinguish a liver with NASH from one with simple steatosis (high TG storage). Insulin resistance increases serum FFA concentrations as a result of failure to suppress HSL-mediated lipolysis in adipose. This has pathophysiologic relevance to VAT stores as: 1) drain directly to the liver, and 2) VAT adipocytes exhibit greater lipolysis and less responsiveness to insulin compared to other peripheral adipose stores.[96-98] These features link centripetal adiposity in humans with MeTS and NAFLD/NASH.

In addition to increased FA uptake and synthesis, impaired VLDL formation/hepatic exportation augments hepatic lipid accumulation in humans with NASH. This implicates dysfunctional VLDL synthesis and secretion as a second distinguishing feature in patients with NAFLD/NASH compared to those with simple steatosis. High-insulin levels associated with MeTS and type 2 diabetes may suppress VLDL secretion and mitochondrial β-oxidation of long-chain FFA, thereby promoting hepatic lipid accumulation. Mitochondrial dysfunction has been implicated in experimental models of NASH associated with abnormal PPAR-α expression.

Insulin Sensitizers: Metformin improves insulin resistance by decreasing hepatic glucose production and increasing skeletal muscle glucose uptake.[12] Further, it reduces hepatic expression of TNF-α, increases FFA oxidation and suppresses lipogenesis through AMPK activation.[12] Yet trials in humans with NAFLD/NASH show improved clinicopathologic markers of liver injury but no significant histologic improvement in steatosis or necroinflammation.[12]

Table 4: Conditions Associated with Severe Hepatic Lipidosis Syndrome in 189 Cats: Cornell University (1990-2010).

	Number		Number
Other Hepatic Disorders	31	*Neoplasia*	29
Cholangitis / Cholangiohepatitis Syndrome	27	LSA	15
Extrahepatic bile duct occlusion	3	Lung Carcinoma	3
Portosystemic vascular anomaly	1	Liver Carcinoma	1
Pancreatitis	21	Adenocarcinoma	5
GI Related	59	pancreatic	2
Inflammatory bowel disease	55	small intestine	1
Peritonitis	5	Carcinomatosis	1
GI Foreign body	2	Osteocondroma	1
Esophageal necrosis/stricture	2	Metastatic: Transitional cell CA	1
Stomatitis	1	*Cardiovascular*	
Chronic diaphragmatic hernia	1	HCM	3
Jejunostomy site sepsis	1	Restrictive CM	1
Intestinal abscess	1	*Hyperthyroidism*	5
Chronic jejunal intussusception	1	*Anemia*	7
Constipation/Obstipation	1	*Neurologic Disease*	4
Diabetes Mellitus	6	*Social interactions in home:*	10
Respiratory Related	6	new pet or house, menacing	
Asthma	2	owner traveling away	
Chylothorax	2	*Miscellaneous*	19
Pleural Effusion	1	Trauma	2
Laryngeal Hemiplegia	1	Steatitis	1
Septicemia	7	Metronidazole toxicity	2
Urologic/Renal Related	7	Hypothyroidism	2
Glomerulosclerosis/Glomerulonephritis	3	Painful tooth	1
Nephritis-ARF	2	Declaw complications	1
Hydronephrosis	1	Cat lost – 1 week	2
Feline Lower Urinary Tract Syndrome	1	Antibiotics: vomiting/anorexia	3
		Trichobezoar or Foreign body	2
		Thrombocytopenia	1
		Chronic FIP	2
		Weight reduction diet-not eaten	5
		Idiopathic-no cause found	5

PPAR-γ Agonists = Thiazolidinedione (TZD) Drugs: PPARγ is plentiful in adipose tissue, where it is essential for adipocyte differentiation and maintenance of normal adipocyte function. PPARγ influences expression of adipokines including aiponectin, leptin and resistin. PPARγ enhances lipogenesis or reduces lipolysis in adipose tissue, reduces lipid content in liver and muscle, increases plasma TG clearance, and improves insulin sensitivity. Increased lipogenesis and enhanced FA oxidation reduces circulating TG and FA by partitioning lipids from insulin response organs to SAT. PPAY-γ enhances insulin sensitivity as reflected in the reduced systemic FA concentrations and effects on hormones, cytokines and proteins influencing insulin responsiveness.

TZD drugs (e.g., pioglitazone) reduce hepatocellular lipid and adipose insulin resistance with benefits mediated via increased adiponectin and the PPAR-γ agonist effect. Reinstating HSL-mediated suppression of lipolysis during fasting, TZDs thwart the unmitigated release of adipose FFA. TZDs also concurrently reduce inflammatory cytokine expression (e.g., TNF-α, IL-6, plasminogen activator inhibitor-1, monocyte chemoattractant protein-1) through inhibition of NF-κβ and nuclear translocation of the glucocorticoid receptor. Several generations of TZDs have been studied with market withdrawal of several following demonstration of severe hepatotoxicity. Pioglitazone and rosiglitazone are approved for use in humans and have significant benefits on metabolic profile, steatosis and necroinflammation but an inconsistent influence on hepatic fibrosis. Unfortunately, relapse of NAFLD has been observed after treatment discontinuation.[12] Darglitazone, studied in healthy, obese cats, was effective in improving insulin sensitivity and glucose and lipid metabolism and lacked toxicity (42-day study).

Feline Hepatic Lipidosis (FHL)

Introduction/Background

Considered the most common form of acute acquired potentially lethal liver disease in the cat, FHL is a syndrome usually observed in inappetent (days to weeks) overconditioned animals. "Idiopathic" FHL is rare, as FHL usually develops secondary to a primary disorder causing inappetence (Table 4).[101] Cats have a propensity for hepatocellular lipid vacuolation that likely reflects unique aspects of feline metabolism as well as the higher fat content of their carnivorous diet and perhaps other

physiologic differences unique to this species. The pure carnivore diet is ample in protein and fat and low in carbohydrates with gluconeogenesis from amino acids sustaining euglycemia. While hepatocellular lipid vacuolation is common in ill or obese cats, the density of vacuolation and extent of cytosolic expansion is considerably less than in FHL where >80% are usually affected. Hepatocytes from cats with FHL may enlarge twofold due to cytosolic TG vacuole distention.[101]

Hepatic Fat in FHL

Hepatic fatty vacuoles in FHL contain neutral fat or TG based on staining with Oil red O using frozen or formalin fixed tissue (paraffin embedded tissue cannot be used). Cats with FHL have >50% of hepatic weight attributable to TG storage and >80% of hepatocytes involved in the process (Figures 2 and 3).[102-104] Characterization of lipid fractions in liver and omental adipose (VAT) in a small number of cats with FHL (n=5) compared to healthy cats (n=5) confirmed greater concentrations of TG in FHL cats, but no unique FA differences. However, omental fat contained high concentrations of palmitate that reconciled with increased concentrations of palmitate found in the liver fat in FHL. This complies with the hypothesis in NAFLD/NASH that hepatic fat reflects FFA derived from VAT. Because the composition of adipose fat reflects the net balance of energy utilization and dietary fat ingestion over time, dietary variables and body composition also need consideration and were unavailable on that data set.[105,106]

Recent study of omentectomy in healthy dogs supports that omental fat indeed reflects VAT as omentectomy significantly increased insulin sensitivity (labeled glucose clamp method).[107] Cats appear to have a greater VAT than humans. Estimation of VAT in cats using computed tomography reported a VAT/SAT ratio in cats of 1.18 ± 0.32 compared to a ratio in humans ranging between 0.19 ± 0.04 and 0.4± 0.3.[108-110] Perhaps a greater proportion of VAT predisposes cats to hepatic steatosis and FHL from simple food abstinence. While observations support that hepatic TG in FHL reflects peripherally mobilized adipose, a relatively diminished rate of hepatic β-oxidation might also promote hepatic fat accretion/retention in this condition. *De novo* fatty acid synthesis in healthy cats occurs primarily from acetate and at much greater rates in adipose tissue than in liver.[111] Considering energy flux in cats with FHL and research data derived from 1) overweight cats undergoing rapid weight loss and 2) experimental induction of FHL, increased *de novo* hepatic lipid synthesis and reduced VLDL exportation are unlikely causes of hepatic lipid accumulation.[112,113] Insufficient increase in the rate of fatty-acid oxidation in face of increased peripheral FA mobilization remains the strongest pathophysiologic scenario.

Clinicopathologic Features (Figure 8):

While cats of any age may be affected by FHL, most are middle-aged, neutered adults (7 [0.5-20] years), have a history of overconditioning (BCS >4/5) and inappetence ranging from two to seven days (>90%) with an ~25% loss body weight (dehydration and body condition).[101,114] On observation, fat is primarily lost from peripheral stores as opposed to abdominal depots. Gastrointestinal signs are common: vomiting ~38% with variable diarrhea or constipation. Other historical features reflect underlying or primary disease processes.[101,114] Jaundice (~70% of cats) and nonpainful, smoothly contoured hepatomegaly are notable on initial physical examination. Cats with severe electrolyte derangements (marked hypokalemia or hypophosphatemia) may demonstrate profound head/neck ventroflexion and ptyalism (reflecting either nausea or hepatic encephalopathy). Severe weakness and recumbency may indicate symptomatic electrolyte disturbances (potassium, phosphate) or thiamine (vitamin B1) deficiency. Cats with neck ventriflexion have limited stress tolerance and may become dyspneic due to ventilatory muscle weakness. These may collapse during routine procedures/restraint or in response to environmental stress (e.g., menacing/barking dogs, aggressive therapeutic interventions).

Because FHL lacks necroinflammatory liver lesions, clinicopathologic features predominantly reflect cholestasis imposed by hepatocyte TG distention, along with body catabolism, fluid, electrolyte and micronutrient depletions and pathologies attributable to underlying or primary disease processes that provoked inappetence.

Common hematologic abnormalities include poikilocytosis and a predisposition to RBC Heinz body formation. Heinz bodies reflect systemic redox imbalance and can develop within hours of exposure to drugs or toxins imposing oxidant challenge (e.g., propofol anesthesia, drugs with a propylene glycol carrier [injectable diazepam, etomidate]). Heinz body hemolysis can lead to sympto-

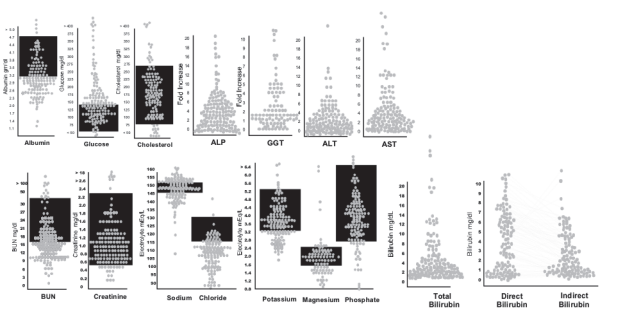


Figure 8. Scattergram showing clinicopathologic features associated with the Feline Hepatic Lipidosis Syndrome in 189 cats. College of Veterinary Medicine, Cornell University, Ithaca, NY.

matic anemia or death. A number of common feline illnesses complicated by FHL, including diabetes mellitus, hyperthyroidism, pancreatitis and necroinflammatory liver disease, also may associate with RBC Heinz body formation.[115] Anemia is identified on initial evaluation in ~22% of cats and may develop during treatment as a result of phlebotomy (blood sampling for tests), hemolysis (Heinz bodies or severe hypophosphatemia provoked by refeeding syndrome), or blood loss (coagulopathy, iatrogenic bleeding from liver biopsy or feeding tube placement). The leukogram reflects underlying disease processes.

Common biochemical features include increased activity of ALT (>72%), AST (>89%), ALP (>80%), subnormal BUN (58%) despite initial dehydration (impaired urea cycle function presumed) and high total bilirubin (variable magnitudes, >95% cats) (Figure 7).[101] There is no diagnostic value in fractionating bilirubin into unconjugated and conjugated forms. Cats with underlying necroinflammatory liver disease (e.g., cholangiohepatitis, major bile duct occlusion, bile duct carcinoma), pancreatitis or pancreatic adenocarcinoma usually develop a fold increase in γGT activity>fold increase in ALP activity.[114] This feature is useful for detecting primary necroinflammatory pathologies involving the hepatobiliary system or pancreas. Nearly 50% of cats with FHL have a >2-fold-greater increase in GT compared to ALP-fold increase implicating co-morbid disorders.[101,116] Concentration of globulins, cholesterol and glucose reflect primary disease processes; few cats present with hypoglycemia. Hyperglycemia evident on presentation in some cats may reflect feline adrenergic stress response or insulin resistance. Influence of overconditioning in cats unaffected by FHL has been shown to increase serum fructosamine; in one study, glycosylated hemoglobin concentrations affiliated with obesity-induced insulin resistance.[117,118] Retrospective study of fructosamine in overconditioned cats identified significantly higher concentrations in male cats compared to female cats (age, weight, BCS matched pairs [n=24]). In the author's hospital population, only ~6% of cats with FHL have insulin-dependent diabetes mellitus, whereas hyperglycemia is noted in ~45% of cats with FHL on presentation. Evaluation of baseline insulin and fructosamine concentrations has not been performed in enough FHL cats to allow speculation regarding insulin resistance in this population. Some cats develop modest to marked increases in creatinine kinase activity reflecting tissue injury associated with intravenous catheter or feeding tube placement, catabolism (muscle mobilization), recumbency or rhabdomyolysis secondary to severe electrolyte depletions (e.g., severe hypophosphatemia).

Electrolyte abnormalities are an important cause of patient morbidity and mortality. Hypokalemia, hypophosphatemia and/or hypomagnesemia are evident on presentation in ~28%, ~14%, ~20%, respectively, with additional depletions appearing after initial crystalloid fluid administration or subsequent to a refeeding syndrome. Severe hypokalemia and hypophosphatemia increase risk for RBC hemolysis (hypophosphatemia), muscle weakness, enteric atony and vomiting, ventroflexion of the head/neck, and neurobehavioral changes that can be confused with hepatic encephalopathy. Hypokalemia imparts a significant risk factor for failure to survive.[114] Vitamin B12 deficiency is common, reflecting inappetence, underlying inflammatory bowel or pancreatic disease, or chronic antimicrobial administration, and may promote development of FHL. Increased unmeasured anions and decreased strong ion difference is consistent with lactate accumulation. Lactate measurements done in a few FHL cats corroborate this hypothesis. Increased lactate concentrations may reflect compromised hepatic metabolism associated with mitochondrial dysfunction or drug administration (e.g., propofol) or thiamine deficiency, known to promote lactic acidosis in humans.[119-123]

Urine sediment usually discloses lipiduria (refractile micro-fat globules in urine) in a buoyant lipid layer; tiny lipid droplets may be confused with coccoid bacteria by the naive observer. Prodigious kaliuresis without glucosuria has been identified in some cats; this may reflect renal tubular lipid lipidosis or an antecedant condition.

Ammonium biurate crystalluria has not been observed or reported in FHL cats lacking a more primary congenital or acquired portosystemic shunt. Experimental modeling of FHL demonstrated ammonia intolerance (4- to 5-fold increase in ammonia concentrations after ammonium chloride challenge). However, test interpretation was confounded by essential amino acid depletions that may have contributed to hyperammonemia (i.e., arginine, methionine).[124,125] Similar amino acid depletions have been shown in primary FHL by the author (unpublished).

Prolonged clotting time is most reliably detected with coagulation tests sensitive to vitamin K deficiency (PIVKA clotting test, Thrombotest, Nycomeda). Prolonged coagulation times respond to parenteral Vitamin K1 administration (three doses of 1.0 mg/kg, IM given at 12-hour intervals).[124]

Hepatic ultrasonography (US) typically discloses subjective hepatomegaly and a diffuse parenchymal echogenicity compared to falciform fat.[127] However, US is heavily operator-dependent with broad interobserver variability. It does not provide quantitative information regarding the degree of lipid accumulation, has low sensitivity for fat infiltration <30% (in humans) and lowest sensitivity in morbidly obese patients (technical difficulties).[128]

Abdominal effusion does not occur as a result of FHL

alone and reflects a more primary disease process or iatrogenic fluid overdosage (i.e., fluid administration based on total body weight in overweight cats). Determination of acute phase markers has not been routinely done. Retrospectively, ~15% have a high fibrinogen and ~15% have a low normal to subnormal fibrinogen perhaps reflecting reduced hepatic protein synthesis.

Circulating Lipids

Serum TGs significantly increase in cats with FHL with greatest distribution in the VLDL fraction (~62% vs. 25% in healthy, lean cats). In one study, the VLDL fraction represented ~19% total lipoprotein mass compared to ~2% in healthy lean cats.[129] Cats with FHL also develop significantly increased concentrations of non-esterified FA (threefold). Characterization of circulating lipids in overconditioned and lean cats in numerous studies and fewer involving FHL have detailed similar trends with FHL cats representing the most severe phenotype.

Cholesterol & Lipid Fractions in FHL and Obese Cats

Serum NEFA, TG, total lipoprotein concentrations, VLDL, LDL and cholesterol concentrations in cats with FHL are significantly higher than normal cats.[112,113,125,129,130] Components of the HDL fractions have been significantly lower than normal cats. While overconditioned cats and obese cats undergoing rapid weight reduction also develop higher cholesterol, TG, VLDL and LDL, and lower HDL fractions compared to lean cats, changes in circulating lipids are most severe in cats with FHL[112,139,140] Collectively, feline lipid profiles in overconditioned cats, in obese cats during weight loss and in FHL cats emulate the dyslipidemia (high cholesterol, high LDL, low HDL) characterized in MeTS and NAFLD/NASH patients. In obese cats, studies have shown that hyperlipidemic changes accompany insulin resistance.

β-*OH Butyrate in FHL*

Ketone body formation is notable in cats with FHL consistent with an enhanced rate of β-oxidation and has been documented in clinical cases and during experimental induction of FHL (offering an unpalatable diet after cats have been fattened). β-OH butyrate concentrations in clinical cases range from mild to marked increases (0.5 to 8.9 mmol/L) comparable to values observed in cats with diabetes mellitus.[133] Experimental induction of FHL confirmed ketone body accumulation as early as the first week of "self-imposed fasting." However, ketone concentrations in experimental FHL have been lower than observed in clinical cases because of early rescue strategies.[124,129,133,Center(unpublished)] Similar levels of ketosis (~5–7 mmol/L) have been shown in humans voluntarily fasting from 1 to 23 days. The longer the fast, the higher the concentration of ketones, as occurs in FHL cats.[134,135]

While finding increased β-hydroxybutyrate concentrations implicates an increased rate of β-oxidation, it remains unknown if β-oxidation is optimally adapted for rapid utilization of large hepatic TG stores. Clinical appraisal of cats with FHL supplemented with L-carnitine along with other nutritional support remarkably improves survival suggesting that suboptimal L-CN (inability to synthesize appropriate amounts?) or other some other micronutrient (B12, B1) may curtail appropriate adjustment of fat oxidation to meet contemporary needs.[101] Demonstration that orally administered L-carnitine can increase fatty-acid oxidation in obese cats suggests that cats may maintain marginal amounts of this conditionally essential nutrient in the face of FHL.[136]

Histologic Features of FHL

Histologic features of FHL resemble simple steatosis in humans rather than NAFLD/NASH. In fact, FHL has morphologic and clinical similarities with hepatic steatosis associated with kwashiork or (severe protein and energy malnutrition) and starvation. Necroinflammatory lesions are absent, lipogranulomas are not observed (during or after syndrome recovery), and there is no zonal distribution of steatosis nor progressive fibrosis.[101,103] Severe cytosolic hepatocyte distention with large fatty vacuoles (macrovesicular) is most typical, reflecting large TG stores within endoplasmic reticulum.[103] However, a subset of cats has both microvesicular and macrovesicular vacuolation (Figure 9). Cytosolic vacuolation often displaces nuclei and organelles to the cell periphery. Cells may be fragile fragmenting on preparation of aspiration or impression smears. Ultrastructural studies have detailed canalicular and sinusoidal compression secondary to marked hepatocyte expansion, structural mitochondrial changes and a paucity of peroxisomes (microbodies).[103,113,124] Mitochondrial numbers

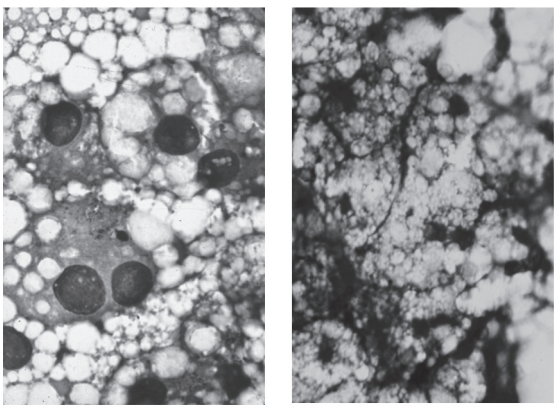


Figure 9

decline with remaining mitochondria larger than normal; this may reflect a change in organelle biogenesis. Disfigured mitochondrial cristae also have been observed implicating metabolic disruption.[103] However, metabolic cellular and gene expression studies have not been conducted to investigate mitochondrial function or gene expression. A reduced number of peroxisomes suggests altered premitochondrial oxidation of very long-chain fatty acids or other peroxisomal functions.[103,113] Yet, since function of peroxisomes is complex, enzymatic and gene expression studies are needed to characterize the physiologic effect of observed organelle changes. Similar morphologic changes in mitochondrial and peroxisomes have been observed in cats during weight gain.[113] Based on ultrastructural features, canalicular compression contributes to the observed cholestasis and reconciles with the altered bile acid pool characterized by HPLC fractionation.[103,137] In addition to markedly increased total serum bile acid concentrations consistent with severe cholestasis, a shift in the fractional bile acid pool composition is characterized by a diminished quantity of secondary bile acids. This change resembles that associated with major bile duct obstruction. Because secondary bile acids are formed by bacterial dehydroxylation in the intestines by enteric organisms, this finding suggests there is a reduced bile acid enterohepatic circulation in FHL.

FHL does not chronically progress to liver failure over months but rather presents as an acute critical syndrome that may culminate in death within a few days or weeks. Clinical features more closely resemble Reye's syndrome, a disorder characterized by diffuse microvesicular hepatic steatosis and liver failure. Reye's syndrome had been most commonly identified in febrile children suffering from viral infections and may involve aspirin-induced mitochondrial toxicity.[138] Finding fine microvesicular hepatocellular vacuolation usually reflects either hepatocellular mitochondrial injury and cell energy deprivation or retention of fatty metabolic products reflecting congenital storage disorders (rare). In Reye's syndrome, fine microvesicular steatosis is linked to mitochondrial injury. Similar microvesicular hepatocyte vacuolation affiliated with increased cell apoptosis or necrosis is observed with certain toxins (e.g., aflatoxicosis).[139] As described previously, this also may develop in some cases of NAFLD/NASH.

Antioxidant Status in FHL

A small number of liver samples from cats with FHL have been analyzed for concentrations of reduced glutathione (GSH) and oxidized glutathione (GSSG) content, normalized against liver protein and DNA. Findings confirmed profoundly depleted total and reduced GSH.[140] This correlates with observed high risk for RBC hemolysis with oxidant challenge (systemic from a primary disease or administration of certain therapeutic products) and subnormal circulating GSH in whole blood.[Center(unpublished)] Presumably, antioxidant depletion reflects nutritional deficiencies as well as increased oxidant challenge imposed by hepatic steatosis (perhaps through mitochondrial dysfunction?) and underlying disease processes as well as systemic inflammation imposed by overconditioned body status. Oxidant injury is implicated in the systemic complications of hepatic steatosis associated with both kwashiorkor and NAFLD/NASH.[141-143]

Inflammatory Adipose Markers: Obese Cats and FHL

Obese cats have greater expression of TNF-α in fat (but not muscle) compared to lean cats (study conducted in weight stable animals [n=24]).[144] TNF-α expression ninefold higher in SAT adipose and twofold higher in muscle was shown in obese cats relative to lean cats; increased IL-6 also has been shown in SAT from obese cats relative to lean cats.[144,145] Increased TNF-α was shown in SAT adipose from obese cats more than a decade ago where adipose from two cats with FHL also was investigated. The FHL cat adipose had markedly increased TNF-α concentrations that rapidly declined with nutritional support (seven days of tube feeding).[146] Finding increased TNF-α expression in fat is consistent with the inflammatory phenotype associated with MeTS in obese humans. Obese cats also have higher plasma α1-acid glycoprotein (acute phase protein) and MCP-1 (acute phase protein and macrophage chemikine) compared to normal conditioned cats.[146] These features are consistent with an inflammatory adipose-driven milieu, as characterized in MeTS, NAFLD/NASH and rodent obesity models. A number of inflammatory mediators complexly influence adipose metabolism (e.g., *in vitro*, TNF-α expression reduces LPL activity) and may ultimately be shown to influence the FHL syndrome.[147]

Does a Neutered Status Facilitate FHL?

Most cats developing FHL are middle-aged and neutered, thus the neutered status of either gender may increase risk for this syndrome. Yet, many pet cats are neutered, introducing bias on this observation. One study characterized adipokines, inflammatory mediators, activity level and adiposity in female DSH cats before and after OVH to shed light on factors predisposing to feline obesity. A sharp acute decline in plasma leptin concentrations immediately followed OVH along with a persistent marked decline in physical activity that coordinated with a tendency to consume more food and

development of an overconditioned status. Twenty-four weeks after OVH (fat mass ~33.5%) increased plasma-fasting glucose, TG and leptin were shown with leptin concentrations highly correlated with increased body mass. Expression studies in SAT disclosed a 23% decrease in leptin, 57% decrease in adiponectin, 53% decrease in LPL, and 38% decrease in HSL. IL-6 expression in SAT increased twofold with TNF-α decreasing by 23%. There were no changes in skeletal muscle mRNA expressions. Because leptin expression in adipose did not increase, an alternative source of plasma leptin was surmised. LPL expression in muscle in this study was discordant with previous work in lean and obese cats where decreased LPL expression in SAT and increased LPL expression in skeletal muscle of obese cats was proposed to favor muscle lipid deposition and insulin resistance.[142] Both studies report reduced LPL activity in SAT that coordinates with production of inflammatory cytokines, (TNF-α[144] and IL-6[145]). Finding decreased adipose HSL expression with increasing adiposity also is at odds with the original feline study[144] of LPL, HSL and HL in overconditioned cats (increased expression in SAT and muscle) but does agree with some work in humans.[146,147] Reduced adipocyte LPL and HSL expression in the OVH study could reconcile with reduced adipocyte metabolic activity associated with gain in adiposity. Estrogen imparts a stimulatory effect on leptin production (note that leptin decreased sharply after OVH before weight gain), which would suppress appetite. Thus, an estrogen-effect stimulating leptin biosynthesis could exist that might be orchestrated through estrogen receptors proven to exist in adipose.[148,149]

Conclusion

A number of investigators have diligently examined overconditioned cats for insulin resistance using a variety of glucose tolerance tests and outcome measures; in-depth consideration of these investigations is beyond the scope of this discussion. However, this body of work confirms that overconditioned cats develop insulin resistance. Proclivity for this scenario may be detected in individual cats when they are in lean body condition (based on demonstration of low-insulin sensitivity).[118,150] Predisposing variables remain unclarified but might also predispose cats to FHL(?).

Evidence is accumulating that cats develop a syndrome similar to human MeTS.[151,152] Obese cats have increased leptin and low adiponectin concentrations and develop insulin resistance (impaired ability of insulin to suppress hepatic glucose output and to promote peripheral glucose disposal).[144,145,150-157] Several investigators have diligently confirmed this phenomenon in overconditioned cats using a variety of glucose challenges and considering both AUC of glucose and insulin as well as calculated ratios. Obese cats have higher TG, cholesterol, VLDL and LDL concentrations compared to normally conditioned cats[112,113,129-132] Yet, the VLDL fractions or incorporated FA are not distinctly unique. There is ample evidence that differences in lipid-partitioning enzymes develop secondary to overconditioning. Overconditioned cats have diminished plasma LPL activity (post-heparin) and both reduced LPL activity and mRNA LPL expression in SAT, while LPL activity and expression in muscle are conserved. However, not all studies confirm identical characterizations.[144] HSL serum activity and expression in SAT and muscle is unaffected by body condition (in one study), dissimilar to reduced HSL expression observed in overconditioned humans. Yet, another study reported reduced adipose HSL expression in obese female cats.[145] Serum HL activity was preserved in overconditioned cats suggesting that HL activity has little specific effect on fat partitioning in obese cats. However, HL has also been shown to be involved in VLDL and IDL-triglyceride metabolism in cats.[159]

Decreased expression of GLUT4 in both muscle and fat develops in chronically obese cats, whereas GLUT4 expression was sustained and enhanced in cats given IV lipid emulsions (x 10 days).[154,156] In humans, reduced GLUT4 expression associates with the early phase of obesity where it is linked with the type 2 diabetic phenotype.[45,46,159-161] Overconditioned cats may increase glycosylated hemoglobin and fructosamine concentrations compared to normal conditioned cats, reflecting insulin resistance.[117, 118] High-glycosylated hemoglobin concentrations have even been provoked by feeding a diet high in saturated fat, similar to the scenario in humans with MeTS. Increased β-OH butyrate concentrations have been documented in overconditioned cats when subjected to a prolonged fast, in the absence of overt diabetes or FHL, as well as in cats with FHL.[124,129,133,Center(unpublished)] Obese cats have been shown to accumulate inflammatory markers in plasma similar to MeTS (TNF-α, MCP-1, α1-acid glycoprotein), to increase TNF-α expression in SAT, and to increase PPAR-γ expression in VAT (lipid infusion model) and expression of inflammatory markers in adipose.[144-146,153,154] Overconditioned cats and cats receiving lipid emulsions (perhaps ill cats, in general, through inflammatory cytokines?) have a propensity for developing hepatic TG vacuolation.[99,111,129,152,160] Overconditioned cats are known to develop asymptomatic diffuse hepatic lipid vacuolation after rapid weight loss (30% over two to five weeks) in the absence of clinicopathologic markers of FHL. Hepatocellular ultrastructural morphology in these cases demonstrate changes similar to those characterized in FHL. Furthermore, short-term iatrogenically increased FA flux (continuous IV in-

fusion of a lipid emulsion) also provokes asymptomatic or symptomatic macrovesicular hepatic steatosis.

Thus, considerable evidence suggests that cats develop components of MeTS including: type 2 DM; increased plasma TG, VLDL and LDL; reduced HDL concentrations, inflammatory cytokines and acute phase proteins in the circulation, SAT or VAT; increased PPAR-γ in VAT; and a propensity for hepatic steatosis. However, the hepatic steatosis and FHL syndrome are profoundly different from NAFLD and NASH. There is no evidence that low-grade hepatic steatosis progresses to a NASH-like syndrome in the cat. FHL represents an acute severe hepatic steatosis associated with compromised hepatic function, but not hepatosteatitis. It has no necroinflammatory component. FHL either resolves with metabolic and nutritional support or the patient succumbs. Relatively few cases of recurrent FHL are documented. Follow-up liver biopsy in cats recovered from FHL have failed to reveal residual lesions, architectural remodelling, fibrosis or lipogranulomas.

Increased flux of TG, FFA and VLDL in overconditioned cats may reflect overnutrition, enhanced hepatic flux of FFA (postprandial, post-absorptive state), and enhanced *de novo* VLDL synthesis (related to increased expression of the main transcription factor of lipogenesis: a sterol-regulatory element-binding protein [Srebp]). However, based on accrued data acquired from metabolic studies of overconditioned cats and cats with FHL, a primary dysregulation of LPL seems unlikely. A pathophysiologic role for LPL activity was an appealing consideration in light of its critical role in lipoprotein metabolism, abundant expression in adipose and muscle, and regulation by insulin and escape during insulin resistance.[99]

Other complicating issues in cats with FHL include vitamin and micronutrient sufficiency. Neuroencephalopathic signs have been related to apparent thiamine deficiency in some patients. A pathologic anion gap/reduced strong ion difference compatible with increased lactate concentrations may implicate a role for thiamine adequacy, mitochondrial dysfunction or drug toxicity. A B12 insufficiency is extraordinarily common in cats with FHL, reflecting primary health problems or chronic antimicrobial administration. Limited B12 may adversely influence the metabolism of methionine through the s'adenosylmethionine transulfuration pathway (recycling of homocysteine back to methionine requires B12 and activates folate) that feeds the transmethylation pathway and provides glutathione (GSH) proven to be low in liver and systemic circulation of cats with FHL.[Center(unpublished)] Yet, insufficient activation of B12 by addition of an adenosy-l or methyl-group (perhaps diminished by a dysfunctional SAMe pathway) and removal of the cyanide moiety (in themitochondria) also may compromise B12 availability. Importantly, measured B12 concentrations do not reflect vitamin functionality (cyanocobalamin also is detected).

There are now four separate investigations that substantiate benefit from L-carnitine on fatty-acid oxidation in overconditioned cats; each of these studies has involved controls for comparative treatment effects. *First*, supplementation of obese cats during a rapid weight loss protocol facilitated weight reduction, increased total CN, acyl-CNs and specifically acetyl-CN.[163] Findings implicated increased fatty-acid oxidation (increased acetyl-CN=last remnant of fatty-acid oxidation) or enhanced urinary acyl-CN elimination. *Second*, preconditioning of overconditioned cats with CN before five weeks of low food intake (feeding an unpalatable diet) protected cats from developing increased circulating FFA and β-OH butyrate concentrations, suggesting that L-CN optimized fatty acid utilization.[133] L-CN might have influenced fatty-acid oxidation in the liver, improved use of ketones in additional tissues (muscle, heart), or facilitated elimination of acy-CN esters in urine. *Third*, the influence of L-CN on fatty-acid metabolism in obese cats was investigated during weight loss using stable isotope labeled fatty acids.[113] While L-CN decreased incorporation of 13C palmitate into VLDL and hepatic TG, it also increased production of β-OH-butyrate. Findings implicated greater partitioning of fat into β-oxidation and less into exported or storage fats. This study also demonstrated a significant reduction in insulin concentrations in L-CN-treated cats that may reconcile with more efficient energy availability. *Fourth*, study of the influence of L-CN on weight loss and resting energy utilization and 13C-palmitate infusions in overconditioned cats demonstrated increased palmitate utilization and stoichiometric calculations, suggesting enhanced fatty acid oxidation.[136] *Fifth*, observational data demonstrates improved recovery in FHL cats supplemented with L-CN, nutritional support and supplementary vitamins compared to cats merely receiving fluid and nutritional support. We can conclude from this collective information that L-CN supplementation is either providing a supraphysiologic dose effect or that pet cats maintain a narrow L-CN surplus when fed formulated feline diets. This may limit adaptive responses appropriate for heightened fatty oxidation for more than a few days. Natural prey of cats contains substantially more L-CN than most feline diets (150 to 3000 mg L-CN/kg animal tissue). However, since L-CN can be synthesized if essential amino acids are available, assumed relative deficiency of L-CN in FHL may reflect complex interactions.

In conclusion, cats with FHL syndrome have many metabolic parallels with MeTS and NAFLD but also several important differences. I see the FHL-affected cat

as having a severe overconditioned phenotype for metabolic maladjustments.

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