LITERATURE REVIEW
ADIPOSE TISSUE

Most supporting tissues contain cells which are adapted for the storage of fat, called adipocytes, which are derived from primitive mesenchyme where they develop as lipoblasts. Adipocytes are found in isolation or in clumps throughout loose supporting tissues or may constitute the main cell type as in adipose tissue (Young B).

The well-documented rise in obesity during the past 30 years has contributed to the negative image of adipose tissue, particularly in the popular mind (Mokdad AH). Adipose tissue was considered as a 'dump yard' of fat; but now we know that it is a vital player in general metabolic process and influences the energy processes of almost all tissues. Adipose tissue, therefore, generally has a rich blood supply. The rate of fat deposition and utilization within adipose tissue is largely determined by dietary intake and energy expenditure, but a number of hormones and the sympathetic nervous system profoundly influence the fat metabolism of adipocytes (Rosen ED). The past two decades, have seen a wave of intense scientific interest in this cell type, fuelled in part by concerns about obesity and its attendant metabolic consequences, and also by the recognition that adipocytes integrate a wide array of homeostatic processes. In addition to regulating fat mass and nutrient homeostasis (discussed below), adipocytes are involved in the immune response, blood pressure control, haemostasis, bone mass, and thyroid and reproductive function. These processes are coordinated mainly through the synthesis and release of peptide hormones by adipocytes (Rosen ED).

It is a well-entrenched fact that adipose tissue is the only organ with unlimited growth potential at any stage of human life. These adipocytes can release protein and lipid derivatives that are highly proangiogenic and have an impact on the preexisting vasculature. Finally, the unique extracellular matrix environment of adipose tissue hosts a number of additional cells such as macrophages and offers unique growth potential for transformed cells such as breast cancer cells (Scherer PE).
Adipocytes store excess energy as fat. A gram of nearly anhydrous fat stores more than six times as much energy as a gram of hydrated glycogen, which is likely the reason that triacylglycerols rather than glycogen were selected in evolution as the major energy reservoir. Triacylglycerols in adipose tissue is derived from three main sources: dietary fat circulating in the bloodstream as chylomicrons; triglycerides synthesized in the liver and transported in blood; and triglycerides synthesized from glucose within adipocytes (Stryer L).

Adipose tissue has an important endocrine role. Through secretion of several biologically active molecules adipocytes modulate energy metabolism and influence general metabolism in coordination with hormones such as insulin to regulate body mass. Adipose tissue is responsible for the secretion of several proteins, collectively known as adipocytokines. These include leptin, adipsin, resistin, adiponectin, tumor necrosis factor alpha, and plasminogen activator inhibitor type 1 (Scherer PE).

There are two main types of adipose tissue:

1) White adipose tissue. This type of adipose tissue comprises up to 20% of total body weight in normal, well nourished male adults and up to 25% in females. It is distributed throughout the body particularly in the deep layers of the skin. In addition to being an important energy store, white adipose tissue acts as a thermal insulator under the skin and functions as a cushion against mechanical shock in such sites as around the kidneys (Young B).

2) Brown adipose tissue. This highly specialized type of adipose tissue is found in newborn mammals and some hibernating animals, where it plays an important part in body temperature regulation. Only small amounts of brown adipose tissue are found in human adults (Young B).
ADIPOKINES

Adipocyte-derived secretory proteins, a group of proteins are referred to as adipokines (Table 1). As the master regulator of systemic lipid storage and through secretion of a number of these adipokines, adipose tissue has an influence on many processes, including energy metabolism, inflammation, and pathophysiological changes such as cancer and infectious disease. Pioneering work from the Spiegelman and Flier laboratories in the mid-1980s highlighted for the first time that adipocytes are an abundant source of a specific secretory protein, called adipisin or complement factor D (Scherer PE). In 1995, Jeffrey Friedman’s (Zhang Y) group identified leptin as a fat cell–specific secretory factor deficient in the ob/ob mouse that mediates the hormonal axis between fat and the brain. Around the same time, Scherer and others described a protein that they initially termed Acrp30, which later became known as adiponectin (Scherer PE). Additional proteins have joined this exclusive club of adipocyte-specific secretory proteins since then, including adipokines such as resistin from Lazer’s lab (Steppan CM) and acylation-stimulating protein (Cianflone K), as well as the recently described visfatin (Fukuhara A) and retinol binding protein-4 (Yang Q). Enzymes such as lipoprotein lipase are also abundantly produced and released from adipocytes. Finally, many proinflammatory cytokines and acute phase reactants originate in the adipocyte. These include alfa-1-acid glycoprotein, serum amyloid A, the C-reactive protein homolog pentraxin-3, the lipocalin 24p3, and a host of cytokines (Scherer PE).

Adiponectin

The hormone adiponectin was identified by several different groups and given various names (for example, apM1, GBP28, AdipoQ and ACRP30). Two types of receptors have been proposed for adiponectin (Rosen ED). Interestingly, adiponectin circulates at extraordinarily high concentrations (5–10 μg ml–1), accounting for 0.01% of all plasma protein. Unlike almost all other adipokines, however, adiponectin levels are inversely correlated with body mass, for obscure reasons. Delivery of adiponectin to obese, diabetic mice stimulates AMP kinase activity in the liver and skeletal muscle, with profound effects on fatty acid oxidation and insulin sensitivity (Rosen ED). Thus adiponectin might play an important role in the
pathogenesis of diabetes and NAFLD. We would discuss adiponectin in detail in next chapter.

**Leptin**

Leptin (also termed OB protein) was discovered in 1994 by Friedman and colleagues (Zhang Y), with the identification of the mutant gene, which underlies the development of the obesity of the *ob/ob* mouse. The identification and sequencing of the ob gene and its product, leptin, in late 1994 opened new insights in the study of the mechanisms controlling body weight and led to a surge of research activity. Currently there is particular interest in the interaction of leptin with other peripheral and neural mechanisms to regulate body weight, reproduction and immunological response (Farid FC). Leptin's involvement in the pathophysiology of obesity, anorexia nervosa, diabetes mellitus, NAFLD, polycystic ovary syndrome, acquired immunodeficiency syndrome, cancer, nephropathy, thyroid disease, Cushing's syndrome and growth hormone deficiency is also being investigated (Trayhurn P).

**Visfatin**

The connection between visceral adiposity and insulin resistance has led several groups to try to identify secreted products derived specifically from this depot. The first such protein reported was visfatin, which had been identified in immune cells years earlier as pre-B-cell colony enhancing factor (PBEF). There were several surprising aspects surrounding this discovery, including the fact that visfatin does not promote insulin resistance — on the contrary, it has a salutary effect on glucose uptake mediated by direct binding and activation of the insulin receptor (Fukuhara A). Visfatin circulates at concentrations well below those of insulin (<10%), however, fasting and feeding do not regulate its expression, making it doubtful that visfatin alone is an important factor in insulin-receptor signaling. Other aspects of visfatin biology require further study. For example, serum levels of visfatin are variably correlated with type 2 diabetes and other insulin-resistant states. Visfatin also has no signal sequence and has been shown to have enzymatic activity as a nicotinamide phosphoribosyltransferase with residence in the nucleus and cytosol. It is not clear whether there is regulated secretion of visfatin or whether serum levels reflect leakage from dead or damaged cells (Rosen ED).
Omentin

Omentin is another peptide secreted predominantly by visceral fat. Like visfatin, it has positive effects on glucose uptake, although omentin works as an insulin sensitizer and does not have insulin-mimetic properties. Unlike visfatin, omentin seems to be made by stromal-vascular cells within the fat pad rather than adipocytes. Interestingly, omentin is produced in considerable quantities by adipose tissue in humans and macaques but not mice (Yang RZ). Omentin’s mechanism of action, including target tissues, a receptor or relevant signal transduction pathways, remains obscure. Tumour necrosis factor-α (TNF-α) was the first secreted adipose protein to be shown to have effects on glucose homeostasis. TNF-α levels are elevated in obesity and in other insulin-resistant states (such as sepsis), addition of TNF-α to cells and mice reduces insulin action, and blockade of TNF-α action by biochemical or genetic means restores insulin sensitivity in vivo and in vitro. Interestingly, TNF-α seems to be derived from cell types other than adipocytes themselves. Macrophages in particular have been implicated in TNF-α production from murine fat pads. Other cytokines, including interleukin-6, are produced by adipocytes, and there is conflicting evidence suggesting that they have both insulin-resistance-promoting and insulin-sensitizing effects. Such ‘adipocytokines’ can promote insulin resistance through several mechanisms. These include c-Jun N-terminal kinase 1 (JNK1)-mediated serine phosphorylation of insulin receptor substrate-1 (IRS-1), IκB kinase (IKK)-mediated nuclear factor-κB (NF-κB) activation, induction of suppressor of cytokine signaling 3 (SOCS3) and production of ROS (Rosen ED).

Tumor Necrosis Factor - α

Tumor necrosis factor-alpha (TNF-α) is a multifunctional cytokine, which exerts a myriad of biological actions in different tissues and species. Many of these actions can perturb the normal regulation of energy metabolism (Trayhurn P). In adipose tissue, in particular, TNF-α has been demonstrated to regulate or interfere with adipocyte metabolism at numerous sites including transcriptional regulation, glucose and fatty acid metabolism and hormone receptor signaling (Rosen ED). Elevated levels of TNF-α have been postulated to induce insulin resistance (Hotamisligil GS) in a variety of catabolic disease states, including cancer, sepsis and trauma. This is supported by the observation that directs exposure of healthy individuals, animals or isolated cells to TNF-α induces a state of insulin resistance. TNF plays significant
role in the development and progression of fatty liver disease through multiple pathways including the induction of oxidative stress [(Medina J), (Sethi JK)].

Resistin

Resistin is a recent addition to the family of adipocytokines. During a search for target genes of TZD, Steppan et al. reported that expression of the gene encoding this protein was suppressed by TZD treatment in adipocytes (Steppan CM). Cultured adipocytes exposed to resistin showed a reduction of insulin stimulated glucose uptake where as the anti-resistin antibody produced the opposite effect. This is important not only as a mechanism of action of TZD but also as a potential general mechanism by which obesity is related to insulin resistance and associated disorders like NAFLD, not to mention the potential therapeutic applications of the discovery (Rosen ED). Population studies have shown polymorphisms in resistin gene in diabetes (Ouchi M).
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<td>List of Adipokines</td>
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<td>Agouti signaling protein</td>
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<td>Angiotensin II</td>
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<td>Complement factor D (adipsin)</td>
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<td>Heparin binding – epidermal growth factor</td>
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<td>Hepatocyte growth factor (HGF)</td>
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<td>Vascular endothelial growth factor (VEGF)</td>
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ADIPONECTIN

Introduction
Adiponectin is the adipocytokine, which has been identified from human fat cDNA. It is a 38kD protein with 247 amino acids and exhibits an adipose-specific expression. Adiponectin exists abundantly in human blood (5-20 μg/ml), and its plasma concentration decreases with fat accumulation in the body. It is the most abundant transcript in human fat. Mouse homolog for adiponectin, Acrp30 and AdipoQ, has been cloned in E.Coli. Plasma adiponectin concentrations are lower in patients with diabetes, nonalcoholic fatty liver disease and ischemic heart disease. (Rosen ED).

<table>
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<th>Synonyms for adiponectin</th>
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<td>30 kDa adipocyte complement-related protein, ACDC, ACRP30, Adipocyte, C1q and collagen domain-containing protein, adiponectin, Adiponectin precursor, AdipoQ, Adipose most abundant gene transcript 1, apM1, APM1, apM-1, APM-1, ATP6A2, ATP6V1A2, GBP28, Gelatin-binding protein, Interferon delta-1 precursor, Isoform HO68, Vacuolar ATP synthase catalytic subunit A, osteoclast isoform, Vacuolar proton pump alpha subunit 2, V-ATPase 69 kDa subunit 2, V-ATPase subunit A 2, VPP2, ADPN (Source: NCBI)</td>
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Adiponectin Identity
Symbol: ADIPOQ
UniProt: Q15848, P37290,
Q58EX9, Q15247
OMIM: 605441
NCBI Gene: 9370
NCBI RefSeq: NP_004788
NCBI RefSeq: NM_004797
NCBI UniGene: 9370
NCBI Accession: BAA86716, D45371
(Source: NCBI)
Adiponectin: Structure, multimeric forms and paralogs

Adiponectin, structurally belongs to the complement 1q family, is composed of four distinct domains: a signal peptide at the N terminus, a short variable region, a collagenous domain, and a C-terminal globular domain homologous to C1q (Figure 1). The crystal structure of adiponectin globular domain reveals a striking resemblance to the structure of tumor necrosis factor (TNF)-α. Adiponectin belongs to a growing family of proteins, all of which contain a C-terminal globular C1q-like domain of about 135 amino acids. Most of them also contain a variable number of “Gly-X-Y” (where X and Y represent any amino acid) collagenous repeats. There are currently 25 proteins, paralogs of adiponectin, belonging to the C1q/TNF-α superfamily (Figure 2). Among these proteins are the Siberian chipmunk hibernating proteins HP20, 25, and 27; serum levels of these three proteins are dramatically reduced during hibernation (Wong GW).

Figure 1
Structure and domains of adiponectin

Adiponectin has an N-terminal collagen-like sequence and a C-terminal globular region. A small fraction of a processed globular form is present in human plasma.
The paralogs [C1q/tumor necrosis factor-a-related proteins (CTRPs) 1–7] were identified by searching GenBank EST and genomic databases with the adiponectin cDNA sequence. The predicted amino acid sequences of all of the CTRPs share a similar modular organization to adiponectin and consist of four distinct domains; a signal peptide (white), a short variable region (grey), a collagenous domain with various length of Gly-X-Y repeats (hatched), and a C-terminal globular domain homologous to complement C1q (black). The predicted signal peptides of mCTRPI, mCTRPI, mCTRPI, mCTRPI, mCTRPI, mCTRPI, and mCTRPI consist of 25, 15, 22, 15, 20, and 16 aa, respectively. The short variable regions of mCTRPI, mCTRPI, mCTRPI, mCTRPI, mCTRPI, mCTRPI, and mCTRPI consist of 73, 24, 22, 14, 58, and 21 aa, respectively. There are a total of 14, 23, 23, 14, and 34 Gly-X-Y (X and Y refer to any amino acid) repeats in the collagenous domain of mCTRPI, mCTRPI, mCTRPI, mCTRPI, and mCTRPI, respectively. The predicted globular domain of mCTRPI, mCTRPI, mCTRPI, mCTRPI, mCTRPI, and mCTRPI consists of 143, 146, 135, 147, 142, and 152 aa, respectively. **·** Indicates cysteine residues; cysteine residues in the signal peptides are not shown because they are not part of the mature proteins. hCTRPs and their corresponding mouse orthologs are highly conserved. The numbers on the right refer to the percent amino acid identity between human and mouse orthologs when comparing the full-length protein (first column), the C-terminal globular domain (second column), or the N-terminal variable region (third column).

Adiponectin is known to form a characteristic homomultimer. It has been demonstrated that simple SDS-PAGE under nonreducing and non-heat-denaturing conditions clearly separates multimeric species of adiponectin (Kadowaki T). Adiponectin in human or mouse serum and adiponectin expressed in NIH-3T3 cells or Escherichia coli forms a wide range of multimers from trimers and hexamers to high molecular weight (HMW) multimers, such as dodecamers and 18mers. Adiponectin can exist as full-length or a smaller, globular fragment; however, almost all adiponectin appears to exist as full-length adiponectin in plasma. A small amount of globular adiponectin was detected in human plasma. It has been proposed that the globular fragment is generated by proteolytic cleavage, and recently it has been shown that the cleavage of adiponectin by leukocyte elastase secreted from activated monocytes and/or neutrophils could be responsible for the generation of the globular fragment of adiponectin. However, the pathophysiological importance of adiponectin cleavage by leukocyte elastase in vivo remains to be determined. Oligomer formation of adiponectin depends on disulfide bond formation mediated by Cys-39. Interestingly, a mutant adiponectin with a substitution of Cys by Ser at codon 39, which formed a trimer and readily underwent proteolytic cleavage, showed much more potent bioactivity, such as reduction of glucose output from primary hepatocytes, than wild-type adiponectin with a HMW. Hydroxylation and glycosylation of the four lysines in the collagenous domain of adiponectin have been shown to play important roles in enhancing the ability of subphysiological concentrations of insulin to inhibit gluconeogenesis in hepatocytes. Adiponectin was reported to be an α-2,8-linked disialic acid-containing glycoprotein, although the biological functions of the disialic acid epitope of adiponectin remain to be elucidated (Kadowaki T). Endogenous adiponectin secreted by adipocytes is posttranslationally modified into eight different isoforms. Carbohydrate detection revealed that six of the adiponectin isoforms are glycosylated. The glycosylation sites were mapped to several lysines (residues 68, 71, 80, and 104) located in the collagenous domain of adiponectin, each having the surrounding motif of GXKGE(D). These four lysines were found to be hydroxylated and subsequently glycosylated. The glycosides attached to each of these four hydroxylated lysines are possibly glucosylgalactosyl groups. Functional analysis revealed that full-length adiponectin produced by mammalian cells is much more potent than bacterially generated adiponectin in enhancing the ability of subphysiological concentrations of
insulin to inhibit gluconeogenesis in primary rat hepatocytes, whereas this insulin-sensitizing ability was significantly attenuated when the four glycosylated lysines were substituted with arginines (Wang Y).

Full-length, but not the globular C-terminal domain of adiponectin produced in mammalian cells, enhanced the ability of insulin to suppress gluconeogenesis and glucose release by primary rat hepatocytes (Wong GW). Differences in activity initially attributed to full-length or globular adiponectin can be ascribed to the different oligomeric forms of adiponectin. As mentioned in serum, adiponectin exists as trimers, hexamers, and high molecular weight species and the proportion of these oligomeric forms changes according to metabolic status and disease states. The higher levels of adiponectin in female subjects are primarily due to increased levels of the HMW complex. A metabolic challenge, such as a glucose or insulin infusion, results in a selective and transient reduction of the HMW form in circulation. The lower-molecular weight hexamer, in contrast, is not affected under those conditions (SchererPE). Different oligomeric forms possess distinct signaling properties; hexameric and high molecular weight forms of adiponectin induce NF-k-B activation, whereas the trimeric forms of adiponectin induce AMP-activated protein kinase (AMPK) activation in muscle (Wong GW). Treatment of mice or patients with various PPAR-g agonists results not only in an improvement in insulin sensitivity but also in a robust increase in circulating adiponectin levels. This increase is primarily due to the induction of the HMW form, if the adiponectin complex distribution is compared before and after treatment in diabetic mice. Type 2 diabetic individuals tend to have lower levels of the HMW form in circulation compared with insulin-sensitive individuals and that the development of type 2 diabetes in an individual is associated with a progressive decrease of the HMW form. The adiponectin sensitivity index (SA) reflects the fraction of adiponectin found in the HMW form. This index has become particularly useful to assess the efficacy of PPAR-g agonist treatment (SchererPE). It should also be mentioned that SA is a good index of hepatic insulin resistance.
Adiponectin Gene

Figure 3

Genomic regions, transcripts, and products

(Source: NCBI Mapviewer)

Figure 4

Genomic context: Chromosome: 3; Location: 3q27

(Source: NCBI Mapviewer)
Adiponectin exerts its effects through its receptors on cell membrane. Two adiponectin receptors (AdipoR1 and AdipoR2) have been found. One comprises two similar transmembrane proteins with homology to G-protein-coupled receptors, known as adipoR1 and adipoR2; a second molecule (T-cadherin) without a transmembrane domain has been proposed to act as a co-receptor for the high-molecular-weight form of adiponectin on endothelial and smooth muscle cells (Rosen ED). AdipoR1 is abundantly expressed in skeletal muscle, brain and other tissues while AdipoR2 is mainly found in liver, also in adipose tissue. The genes encoding these receptors are located on different chromosomes, and the gene products constitute a novel class of seven-transmembrane domain receptors. Yamauchi et al. isolated cDNAs encoding ADIPOR1 and ADIPOR2 by expression cloning (Yamauchi T). The mouse Adipor1 protein contains 375 amino acids and has a predicted molecular mass of 42.4 kD. Human ADIPOR1 also has 375 amino acids and shares 96.8% identity with the mouse protein. ADIPOR1 and ADIPOR2 are highly related structurally, and mouse Adipor1 and Adipor2 share 66.7% identity. ADIPOR1 and ADIPOR2 are 7-transmembrane domain proteins, but they are structurally, topologically, and functionally distinct from G protein-coupled receptors (GPCRs). Epitope tag labeling showed that the N terminus is internal and the C terminus is external in the ADIPORs, a topology opposite that of GPCRs. ADIPOR1 and ADIPOR2 are conserved from yeast to human, especially in the membrane-spanning regions. The yeast homolog has a principal role in metabolic pathways that regulate lipid metabolism, such as fatty acid oxidation. Northern blot analysis of mouse or human tissues detected ubiquitous expression of a major 2.0-kb ADIPOR1 transcript, with highest expression in skeletal muscle (Kadowaki T). Besides ADIPOR1 & ADIPOR2 adiponectin also bind to, previously undocumented adiponectin receptor, calreticulin, expressed on the surface of macrophages. Here adiponectin acts as a bridge molecule between macrophages that remove cellular debris (Takemura Y).
Adiponectin: Mechanism of Action

The immediate downstream signaling events are largely unknown, nevertheless stimulation of the receptors has been shown to activate AMP-activated protein kinase (AMPK) and induce peroxisome proliferator–activated receptor -a (PPAR-a) signaling (Bjursell M). In parallel with its activation of AMPK, adiponectin stimulates phosphorylation of acetyl coenzyme-A carboxylase (ACC), fatty-acid combustion, glucose uptake, and lactate production in myocytes, and also stimulated phosphorylation of ACC and caused a reduction in molecules involved in gluconeogenesis in the liver, which can account for the acute glucose-lowering effects of adiponectin in vivo (Kadowaki T). The mechanism for AMPK activation by adiponectin remains unclear, and in particular, it is not known whether they act by increasing the AMP: ATP ratio or via some more novel mechanism. Yeast two-hybrid cDNA library (derived from human fetal brain) screening, using the cytoplasmic domain of AdipoR1 as bait, led to the identification of a phosphotyrosine binding domain and a pleckstrin homology domain-containing adaptor protein, APPL1 (adaptor protein containing pleckstrin homology domain, phosphotyrosine binding (PTB) domain and leucine zipper motif). APPL1 interacts with adiponectin receptors in mammalian cells and the interaction is stimulated by adiponectin. Overexpression of APPL1 increases, and suppression of APPL1 level reduces, adiponectin signaling and adiponectin-mediated downstream events (such as lipid oxidation, glucose uptake and the membrane translocation of glucose transport 4 (GLUT4). Adiponectin stimulates the interaction between APPL1 and Rab5 (a small GTPase), leading to increased GLUT4 membrane translocation. APPL1 also acts as a critical regulator of the crosstalk between adiponectin signaling and insulin signaling pathways (Mao X).

Male but not female AdipoR1-/- mice had increased body weight gain in spite of food intake similar to that of wild-type controls. Furthermore, male AdipoR1-/- mice had increased total body fat mass at 15 weeks of age, as determined by DEXA (Bjursell M). Male AdipoR1-/- mice had decreased glucose tolerance as determined by oral glucose tolerance testing. AdipoR1 deficiency did not affect plasma triglyceride levels, but plasma cholesterol levels were elevated in AdipoR1-/- females compared with wild-type controls. Liver triglyceride content tended to be
increased in AdipoR1/- mice compared with wild-type controls. The expression of PPARα mRNA was unchanged, and its downstream target gene carnitine palmitoyl transferase-1 (CPT-1)α was increased in AdipoR1/- livers compared with wild-type controls, while PPARα or CPT-1α mRNA levels did not differ between the genotypes in skeletal muscle. In addition, AdipoR1/- mice did not show changed phosphorylation of AMPK in liver, skeletal muscle, or heart. This suggests that AdipoR1 -/- mice do not have decreased glucose tolerance as a result of changed PPARα activity or basal AMPK phosphorylation. AdipoR1/- deficiency results in higher expression of AdipoR2 in liver and BAT but not in WAT, skeletal muscle, or brain. AdipoR1/- mice had decreased energy expenditure. Male and female AdipoR2/- mice were lean on regular chow and resistant to high fatty diet induced weight gain despite similar or increased food intake. After 14 weeks of high fat diet (HFD), female AdipoR2/- adipocytes in WAT were smaller in size and their BAT contained less number of vacuoles than wild-type controls. Testes weight was found to be reduced in AdipoR2/- males compared with wild-type controls. This weight reduction was associated with an atrophy of the seminiferous tubules and aspermia but no significant change in plasma testosterone levels. Interestingly, both male and female AdipoR2/- mice had higher brain weight compared with wild-type controls. On HFD, female AdipoR2/- mice had significantly lower (76%) fasting insulin levels and lower insulin and glucose response following an oral glucose tolerance test. AdipoR2/- mice had reduced total plasma cholesterol levels irrespective of diet, while plasma triglyceride levels were unchanged. The HDL, apolipoprotein A1, hepatic ATP binding cassette transporter-1 (ABCA1) mRNA levels were decreased. AdipoR2/- mice had decreased AdipoR1 mRNA levels in liver and BAT. The mRNA expression of PPARα and CPT-1β were down regulated in AdipoR2/- livers, while AdipoR2/- mice and wild-type controls had similar levels of PPARα and CPT-1β in skeletal muscle. Thus, the phenotype of the AdipoR2/- mice is similar to that observed following adiponectin treatment. Adiponectin operates as a key regulator of adipocyte secretory function. This autocrine action may prevent the induction of skeletal muscle insulin resistance and may partly explain the antidiabetes action of this hormone (Bjursell M).

Circulating adiponectin concentrations are lower in association with obesity and insulin resistance, both of which are frequently accompanied by chronic increases in
circulating interleukin-6 (IL-6) and (or) tumor necrosis factor-a (TNFα). Adiponectin suppresses activation of the nuclear factor kappa B (NFκB) transcription factor in aortic endothelial cells and porcine macrophages. Adiponectin was also shown to inhibit TNF-a-induced NF-κ-B activation through the inhibition of IκB phosphorylation (Kadowaki T).

Interestingly, in skeletal muscle, adiponectin increased expression of molecules involved in fatty-acid transport such as CD36, in combustion of fatty-acid such as acylcoenzyme A oxidase, and in energy dissipation such as uncoupling protein 2. Adiponectin decreases triglyceride content in skeletal muscle which in turn contributes to improvement in insulin action (Kadowaki T).

Prolonged treatment with adiponectin in MDA-MB-231 cells (breast carcinoma cell line) blocked serum-induced phosphorylation of Akt and glycogen synthase kinase-3B (GSK-3B), suppressed intracellular accumulation of B-catenin and its nuclear activities, and consequently reduced expression of cyclin D1. The inhibitory role of adiponectin on MDAMB-231 cell growth might be attributed to its suppressive effects on the GSK-3B/B-catenin signaling pathway (Wang Y).

Adiponectin can bind to the globular domain of the A chain of collagenase-digested C1q, and C1q binding induced deposition of C4 and C3 through activation of the classical complement pathway. This suggests that adiponectin is member of the pattern-recognition family of defense collagens, able to bind target molecules and activate complement. It may therefore play an important role in innate immunity and autoimmune phenomena (Peake PW).

Globular adiponectin (gAd) strongly inhibited TNF-a/RANKL-induced differentiation of osteoclasts by interfering with TNF receptor-associated factor-6 production and calcium signaling; consequently, the induction of nuclear factor of activated T cells c1 (NFATc1) was strongly inhibited. Moreover, we observed that inhibition of AMP-activated protein kinase abrogated gAd inhibition for TNF-a/RANKL-induced NFATc1 expression. Thus it is possible that adiponectin acts as a potent regulator of bone resorption observed in diseases associated with cytokine activation (Yamaguchi N).
Pressure overload in adiponectin deficient mice resulted in enhanced concentric cardiac hypertrophy that was related with increased extracellular signal-regulated kinase (ERK) and diminished AMPK signaling in myocardium. This means adiponectin inhibits hypertrophic signaling in myocardium through AMPK signaling. Thus adiponectin may have a utility in treating cardiomyopathy which is associated with is diabetes (Shibata R).

It is interesting to note that human placenta and adipose tissue have been reported to produce and secrete various proinflammatory factors including cytokine such as leptin, resistin, adiponectin, IL-1β, IL-6, TNFα, and prostaglandins (PGs) such as PGE2 and PGF2α. It may be remembered that there is physiological insulin resistance in pregnancy. Both leptin and adiponectin significantly increased PGF2α release from human placenta; there was no effect of these hormones on PGF2α release from adipose tissue. Leptin- and adiponectin-induced proinflammatory response could be abrogated by treatment with the anti-inflammatory ERK1/2 MAPK inhibitor U0126, the troglitazone (PPAR-γ agonist), and the nuclear factor-kB inhibitor BAY 11-7082. Leptin and adiponectin activate proinflammatory cytokine release and phospholipid metabolism in human placenta and adipose tissue, and anti-inflammatory agents can abrogate leptin- and adiponectin induced inflammation (Lappas M).

[Note on AMPK: All cells must maintain a high ratio of cellular ATP: ADP to survive. Because of the adenylate kinase reaction (2ADP ↔ ATP + AMP), AMP rises whenever the ATP: ADP ratio falls, and a high cellular ratio of AMP: ATP is a signal that the energy status of the cell is compromised (Hardie GD). The AMP-activated protein kinase (AMPK) is the downstream component of a protein kinase cascade that is switched on by a rise in the AMP: ATP ratio, via a complex mechanism that results in an exquisitely sensitive system. AMPK is switched on by cellular stresses that either interferes with ATP production (e.g. hypoxia, glucose deprivation, or ischemia) or by stresses that increase ATP consumption (e.g. muscle contraction). It is also activated by hormones that act via Gq-coupled receptors, and by leptin and adiponectin, via mechanisms that remain unclear. Once activated, the system switches on catabolic pathways that generate ATP, while switching off ATP-}
consuming processes that are not essential for short-term cell survival, such as the synthesis of lipids, carbohydrates, and proteins (Hardie GD). Population studies have shown that AMPKα2 subunit polymorphism is associated with diabetes (Horikoshi M).

Regulation of Adiponectin

Adiponectin gene regulation includes a number of hormonal and environmental factors. Adiponectin gene expression in white adipose tissue is decreased by obesity, glucocorticoids, β-adrenergic agonists and TNF-α and increased by leanness, cold exposure, adrenalectomy and IGF-1 (Haluzik M). The administration of thiazolidinediones (TZDs), which are Peroxisome Proliferator Activator Receptor-γ (PPAR-γ) agonists, significantly increased the plasma adiponectin concentrations in insulin resistant humans and rodents without affecting their body weight. Adiponectin mRNA expression was normalized or increased by TZDs in the adipose tissues of obese mice. In cultured 3T3-L1 adipocytes, TZD derivatives enhanced the mRNA expression and secretion of adiponectin in a dose- and time-dependent manner. Furthermore, these effects were mediated through the activation of the promoter by the TZDs (Kadowaki T). On the other hand, TNF-α, which is produced more in an insulin-resistant condition, dose-dependently reduced the expression of adiponectin in adipocytes by suppressing its promoter activity. TZDs restored this inhibitory effect by TNF-α. TZDs act via PPAR-γ to increase the transcription of adiponectin or decrease the expression of TNF-α. Glucocorticoids reduces adiponectin secretion but TNF-α don't have any direct effect on adiponectin expression on cultured adipocytes (Yamauchi MD). GH increases adiponectin gene expression through the JAK2-P38 MAP kinase pathway, and that elevation of adiponectin production might represent a novel mechanism by which GH regulates systemic energy metabolism and insulin sensitivity. The promoter region of Apm1 contains consensus sequences for PPARγ, LRH binding (Ikeda K). Several transcription factors regulate adiponectin gene expression such as PPAR-γ, liver receptor homolog-1 (LRH-1), CCAAT/enhancer binding protein (C/EBP), nuclear transcription factor-Y (NF-Y), SREBP-1c, NFATc4 and ATF3, [(Iwaki M), (Ikeda K), (Kim HB)]. Nuclear factor of activated T-cells (NFATc4) and activating transcription factor 3 (ATF3) function as negative regulators of adiponectin gene
expression, and may play critical roles in down regulating adiponectin expression in obesity and type-2 diabetes (Kim HB). SIRT1 Regulates Adiponectin Gene Expression through Foxo1-C/Enhancer-binding Protein-α Transcriptional Complex. Foxo1 interacts with CCAAT/enhancer-binding protein -α (C/EBP-α) to form a transcription complex at the mouse adiponectin promoter and up-regulates adiponectin gene transcription (Qiao L). Foxo1 modulates energy homeostasis in WAT and BAT through regulation of adipocyte size and adipose tissue-specific gene expression in response to excessive calorie intake (Nakae J). Oncostatin M but not IL-6 decreases adiponectin expression and induce dedifferentiation of adipocytes by JAK3- and MEK-dependent pathways. Insulin-stimulated Akt activity in adipocytes may play an important role in the regulation of adiponectin secretion (Songa HY).

**Adiponectin and Diseases:**

Hypoadiponectinemia is associated with obesity, diabetes mellitus type 2, cardiovascular mortality, NAFLD, polycystic ovarian disease and gestational diabetes. Recent years witnessed an explosion of publications on adiponectin. A PubMed query returned 4054 items on adiponectin on 13th June 2008. Studies have come connecting adiponectin to life span, circadian rhythm, neuronal signaling, hypoxia, oxidant stress, reperfusion injury, myocardial infarction, bariatric surgery, end stage nephropathy, migraine, degree of systemic wasting that precedes death, epithelial ovarian tumors, breast cancer, lung cancer, liver cancer, Barrett’s Esophagus, colorectal cancer, renal cell cancer, AIDS, Hepatitis C, Hepatitis B etc (PubMed query results).

**Role of Adiponectin in Metabolic Syndrome**

Metabolic Syndrome is group of cardio-vascular metabolic risk factors which include obesity, hypertension, and dyslipidemia and glucose intolerance. Plasma adiponectin is decreased in MS (R. Lindsay). Adiponectin improves glucose tolerance. It activates insulin receptor substrate-1 (IRS-1) mediated phosphatidylinositol-3 kinase (PI-3K) and glucose uptake in skeletal muscle cells, enhances muscle [beta]-oxidation via the activation of AMP-kinase, and suppresses hepatic glucose production (Gil-Campos M). Adiponectin has recently been shown
to be a promising candidate for the treatment of obesity–associated metabolic syndromes. Delivery of recombinant adiponectin into mice dramatically alleviated hepatomegaly and steatosis and also significantly attenuated inflammation and elevated levels of serum ALT. This raises the possibility of using adiponectin as a potent drug in the treatment of NAFLD and NASH. Replenishment of adiponectin in mice can decrease hypertension, reverse insulin resistance, and cause sustained weight loss without affecting the food intake (Xu A). It is known that fat rich; glucose rich diet markedly decreases the transcription of adiponectin gene (Naderali EK).

Early studies using euglycemic clamp techniques indicated that adiponectin, when injected in a recombinant form, has a profound impact on the liver. Specifically, it reduces the need to infuse glucose by enhancing the insulin-mediated repression of glucose production in the liver. Similar observations can be made in vitro using primary hepatocytes exposed to varying levels of insulin in the presence or absence of adiponectin. Increasing levels of insulin repress the glucose output of hepatocytes (Scherer PE). The presence of adiponectin at physiological concentrations maximizes the insulin effects even at low concentrations, suggesting that adiponectin functions as a highly effective insulin sensitizer at the level of the liver (Scherer PE). Despite the fact that adiponectin is produced exclusively in adipocytes, its serum levels tend to be lower in patients with increased fat mass. Some of the best correlations can be seen with insulin sensitivity, whereby higher levels of serum adiponectin are associated with increased insulin sensitivity. Patients with cardiovascular disease and other states associated with increased inflammation tend to have decreased levels of adiponectin. Consistent with the improved insulin sensitivity generally observed in female compared with male subjects, adiponectin levels are higher in women than in men. Patients with type 2 diabetes and other diseases associated with reduced insulin sensitivity, such as generalized or HIV induced lipodystrophies, have decreased levels of adiponectin (Scherer PE). Frequently, decreased adiponectin levels are not only observed in association with type 2 diabetes and cardiovascular disease but also serve as powerful predictors for the future development of these syndromes even in the absence of any other manifestations of the disease. The region around 3q27 encoding the adiponectin locus has been identified as a susceptibility region for MS, and many studies have
associated mutations and polymorphisms in the gene encoding adiponectin with an increased prevalence of diabetes (Stumvoll M). Finally, other than weight loss, the only other viable approach to significantly improve adiponectin levels is through the use of pharmacological activators of the nuclear receptor peroxisome proliferator–activated receptor (PPAR)-g by thiazolidinediones (TZDs), which are widely used as insulin sensitizers in diabetes clinic. These studies are highly suggestive of a direct involvement of adiponectin in mediating the improvements in insulin sensitivity induced by PPAR-g agonists (Scherer PE). A mouse that overexpresses adiponectin from an adipose tissue–specific promoter showed modest overexpression in the same physiological range that can be achieved with PPAR-g agonist treatment. These mice display increased hepatic insulin sensitivity and are resistant to the negative physiologic impact of a high-fat Western diet. They also show substantial improvements in their lipid profile. These and many additional effects seen in the mice are similar to effects that can be achieved by prolonged treatment with PPAR-g agonists. In addition, just like during PPAR-g agonist treatment, magnetic resonance imaging reveals a significant increase in adipose tissue mass, particularly in the interscapular region, which contains brown adipose tissue, the tissue responsible for nonshivering thermogenesis (Scherer PE).

Moderate overexpression of adiponectin in the classical ob/ob mouse, which is deficient in functional leptin results in a complete normalization of metabolic parameters. There were dramatic improvements in glucose clearance during an oral glucose tolerance test, improvements in fasting insulin levels, islet morphology, and b-cell function. The ob/ob mice over expressing adiponectin display a more efficient clearance of triglycerides during lipid ingestion, likely mediated at least in part through higher levels of lipoprotein lipase in all fat pads examined. They have a decreased deposition of lipids in the liver. A closer look at the adipose tissue of these mice shows that most of the adipocytes are considerably smaller in these mice, consistent with the widespread believe in the field that smaller adipocytes have a more positive impact on the metabolic profile than larger adipocytes (Scherer PE).

‘ob/ob’ mice overexpressing adiponectin are markedly bigger than their ob/ob littermates. This increase in body weight is mostly due to an increase in total adipose tissue mass. Adiponectin-mediated weight gain can be augmented even further. If a
more active version of adiponectin (a protein carrying a mutation at position cys39) is expressed as a transgene at very low steady-state levels, we obtain massively obese mice, weighing more than four times the weight of their wild-type littermates. Compared with ob/ob littermates, they are nearly twice as heavy. Again, despite this massive obesity, these mice are metabolically remarkably healthy, with reduced circulating lipids and a reduced accumulation of lipids in the liver. Considering these facts adiponectin can be considered as a starvation signal produced and released from the adipocyte, mediating a redistribution of lipid deposition away from tissues such as liver and muscle into adipose tissue where these triglycerides can be stored in a more inert fashion. As a result, decreased lipid level in muscle and particularly in the liver, causes improvement in insulin sensitivity (Scherer PE).

Overall, the adiponectin-mediated redistribution of triglycerides is remarkably similar to the actions of PPAR-γ agonists. The proposed mechanism of action of these insulin-sensitizing compounds rely to a large extent on the ability to redistribute triglycerides to adipose tissue and to partition triglycerides within adipose tissue into an increased number of smaller adipocytes. Models of adiponectin overexpression have provided meaningful insights into transgene-mediated chronic overexpression of adiponectin in the obese state, triggering a lipid deposition in adipose tissue. Lack of adiponectin leads to insulin resistance, particularly in the context of high-fat diet. ob/ob mice that lack adiponectin display a reduced response to PPAR-γ agonist treatment. Similar observations can be seen for the activation of AMP-activated protein kinase (AMPK) in the liver, an important local mediator of PPAR-γ agonist action. Wild-type mice respond to PPAR-γ agonists through a marked induction of AMPK activity. The response in adiponectin-null mice is sharply reduced. However, even though the induction of adiponectin is an intrinsic component of the mechanism of action of PPAR-γ agonists, it is clear that additional adiponectin- and AMPK independent mechanisms are contributing to PPAR-γ agonist effects (Scherer PE).

Adiposity, particularly increased intra-abdominal fat, is a predisposing factor for the development of insulin resistance in obesity and type 2 diabetes. Visceral fat seems to differ from subcutaneous adipose tissue in adipocytokine production. In Zucker fatty versus lean rats, visceral fat, as opposed to subcutaneous fat, exhibited
relatively higher levels of adiponectin production in lean animals. These results suggest that an impaired depot-specific expression of adiponectin is a contributing factor for the development of insulin resistance (Altomonte J). In summary, adiponectin has gained widespread acceptance as a marker in the context of obesity and diabetes. Increased evidence points to an involvement in cardiovascular disease. Due to the impact of inflammation on adiponectin levels, adiponectin is emerging as an important link among type 2 diabetes, cardiovascular disease, and the metabolic syndrome.

**Adiponectin gene mutations are associated with metabolic syndrome**

Genome wide scan studies have mapped a diabetes susceptibility locus on chromosome 3q27, where the adiponectin gene is located. A Japanese group (2000) identified two nucleotide changes in the adiponectin gene, a G/T polymorphism at nucleotide 94 in exon 2 was associated with neither plasma adiponectin concentrations nor the presence of obesity; however, the missense mutation R112C showed markedly low plasma adiponectin concentration (Takahashi M). The same group (2002) studied four missense mutations (R112C, I164T, R221S, and H241P) in the globular domain. Among these mutations, the frequency of I164T mutation was significantly higher in type 2 diabetic patients than in age and BMI-matched control subjects (Kondo H). Furthermore, plasma adiponectin concentrations of subjects carrying I164T mutation were lower than those of subject’s without the mutation. All the subjects carrying I164T mutation showed some features of metabolic syndrome, including hypertension, hyperlipidemia, diabetes, and atherosclerosis. These findings suggest that I164T mutation is associated with low plasma adiponectin concentration and type 2 diabetes. A study by Kazuo Hara et al provides further evidence that adiponectin is a novel susceptibility gene for type 2 diabetes. They found SNP 276 to be associated with type 2 diabetes. SNP276 is an intron of the adiponectin gene. Subjects with G/G genotype are at risk a higher risk of developing Type 2 diabetes (Hara K). [(Stumvoll M), (Takahashi M), (Kondo H), (Hara K), (Mori Y)]

There is a paucity of studies from India on the genetics of Diabetes type 2 from the Indian subcontinent. In a recent study (2008, July) from Chennai, South India, 2,000
normal glucose tolerant (NGT) and 2,000 type 2 diabetic, unrelated subjects were examined. Serum adiponectin levels were measured and subsequent SNP analysis identified two proximal promoter SNPs (−11377C→G and −11282T→C), one intronic SNP (+10211T→G) and one exonic SNP (+45T→G). Logistic regression analysis revealed that subjects with TG genotype of +10211T→G had significantly higher risk for diabetes compared to TT genotype but no association with diabetes was observed with GG genotype. Stratification of the study subjects based on BMI showed that the odds ratio for obesity for the TG genotype was 1.53 and that for GG genotype was 2.10. Among NGT subjects, the mean serum adiponectin levels were significantly lower among the GG and TG genotypes compared to TT genotype. Among Asian Indians there is an association of +10211T→G polymorphism in the first intron of the adiponectin gene with type 2 diabetes, obesity and hypoadiponectinemia (Vimaleswaran KS 2008).

Adiponectin and Nonalcoholic Fatty Liver Disease

Delivery of recombinant adiponectin into these mice dramatically alleviated hepatomegaly and steatosis and also significantly attenuated inflammation and the elevated levels of serum alanine aminotransferase (Xu. A). NAFLD in man is associated with hypo-adiponectinemia but its significance is not well established. Studies link hypoadiponectinemia with NAFLD in adults and children and, in particular, with necroinflammatory NASH (Lonardo A). Adiponectin modulates lipid metabolism and liver injury in nonalcoholic fatty liver disease (NAFLD) even in the absence of obesity, dyslipidemia, and diabetes (Musso G). Some, but not all, studies have characterized low serum adiponectin levels as a factor that distinguishes NASH from simple steatosis (Farrell GC).

Weight loss and PPAR-γ agonists form the first line management of NAFLD. Similarly other than weight loss, the only other viable approach to significantly improve adiponectin levels is through the use of pharmacological activators of PPAR-γ by TZDs, which are widely used as insulin sensitizers in diabetes clinic. This shows the link between adiponectin and NAFLD. It is possible that PPAR-γ agonists at least partly exerts its effect by up regulating adiponectin. Several studies including our study have demonstrated that serum adiponectin level goes down in NAFLD. Adiponectin gene mutations are associated with NAFLD. The 45TT and
276GT/TT adiponectin genotypes were more prevalent in NAFLD patients than in controls and independently predicted the severity of liver disease in NASH. In both patients and controls, these genotypes exhibited a blunted postprandial adiponectin response and higher postprandial triglycerides, free fatty acids, oxidized LDL, and VLDL levels than their counterparts, despite comparable fasting adipokines, lipids, dietary habits, adiposity, and insulin resistance (Musso G, 2008).

**Adiponectin: atherosclerosis and Inflammation**

Low plasma adiponectin concentration is now recognized to be a new potential risk factor of cardiovascular morbidity (Szmitko PE). The experimental studies indicate that inflammatory processes may modulate adiponectin secretion. TNF-α and IL-6 inhibit adiponectin gene expression in cultured adipocytes. In general population, an inverse relationship was found between plasma concentrations of adiponectin and C-reactive protein (CRP) (Chudek J). In apolipoprotein E deficient mice, infection of adenovirus-expressing human adiponectin ameliorates atherosclerosis (Okamoto Y).

Finally, adiponectin deficiency in knockout mice causes diet-induced insulin resistance and diabetes and increases neointimal thickening in the arterial wall by mechanical injury. Adiponectin supplement completely reverses the diabetic and atherosclerotic phenotypes of the knockout mice [(Okamoto Y), (Kubota N)].

Adiponectin exhibits anti-inflammatory action and it exerts anti-atherogenic effects in tissue cultures. Adiponectin suppresses monocyte adhesion to endothelial cells by reducing the nuclear factor-κB (NF-κB) signaling and the mRNA expression of adhesion molecules in endothelial cells (Ouchi N, 2000). Adiponectin inhibits foam cell formation from macrophages by reducing the expression of class A macrophage scavenger receptor and lipid accumulation (Ouchi N, 2001). It also suppresses the proliferation and migration of vascular smooth muscle cells by reducing the effects of various growth factors, including platelet-derived growth factor (PDGF)-AA, PDGF-BB, and heparin-binding epidermal growth factor (HB-EGF), on the cells (Arita Y). Adiponectin also decreases expression of adhesion molecules (VCAM-1; ICAM-1, E-selectin) in endothelial cells in response to inflammatory stimuli, such as tumor necrosis factor-a (TNF-a) and finally attenuates neointimal proliferation (Ouchi N 1999).
The vascular action of insulin to stimulate endothelial production of nitric oxide (NO), leading to vasodilation and increased blood flow is an important component of insulin-stimulated whole body glucose utilization. This endothelium-dependent mechanism entails the stimulation of nitric oxide production by adiponectin (Chen H). Endothelium-dependent vasorelaxation was impaired in subjects with low plasma adiponectin concentration. Thus, it has been suggested that low plasma adiponectin concentration might be an important mechanism linking obesity with hypertension and atherosclerosis. The antiatherogenic effect of adiponectin was confirmed in ApoE-deficient mice, a well-known model of spontaneous atherosclerosis (Okamoto Y).

Adiponectin suppresses production of cytokines, like TNF-α, by macrophages, suppresses accumulation of lipids in monocyte-derived macrophages and inhibits transformation of macrophages into foam cells (Szmitko PE). It also inhibits cell proliferation stimulated by oxidized LDL (Motoshima H). Vascular inflammation and subsequent matrix degradation play an important role in the development of atherosclerosis. Adiponectin accumulates to an injured artery and attenuates vascular inflammatory response. Adiponectin participates in the stabilization of atherosclerotic plaques by increasing expression of tissue inhibitor of metalloproteinase-1 (TIMP-1) in infiltrating macrophages (Kumada M).

Adiponectin inhibits TNF-α–induced expression of endothelial adhesion molecules and that plasma adiponectin level was reduced in patients with coronary artery disease (Ouchi N 1999). Adiponectin modulates the inflammatory response of endothelial cells through cross talk between cAMP-PKA and NF-kB signaling pathways (Ouchi N 2000).

Adiponectin facilitates the uptake of early apoptotic cells by macrophages, an essential feature of immune system function. Adiponectin-deficient (APN-KO) mice were impaired in their ability to clear apoptotic thymocytes in response to dexamethasone treatment, and these animals displayed a reduced ability to clear early apoptotic cells that were injected into their intraperitoneal cavities. Conversely, adiponectin administration promoted the clearance of apoptotic cells by macrophages in both APN-KO and wild-type mice. Adiponectin overexpression also
promoted apoptotic cell clearance and reduced features of autoimmunity in lpr mice whereas adiponectin deficiency in lpr mice led to a further reduction in apoptotic cell clearance, which was accompanied by exacerbated systemic inflammation. Adiponectin was capable of opsonizing apoptotic cells, and phagocytosis of cell corpses was mediated by the binding of adiponectin to calreticulin on the macrophage cell surface. Adiponectin protects the organism from systemic inflammation by promoting the clearance of early apoptotic cells by macrophages through a receptor-dependent pathway involving calreticulin (Takemura Y).

Hypoxia, Ischemia, oxidant stress, angiogenesis, nitric oxide and adiponectin

Low plasma levels of adiponectin (hypoadiponectinemia) and elevated circulating concentrations of plasminogen activator inhibitor (PAI)-1 are causally associated with obesity-related insulin resistance and cardiovascular disease. Hypoxia and reactive oxygen species dysregulates the production of adiponectin and plasminogen activator inhibitor-1. The dysreguation of adiponectin by hypoxia is independent of reactive oxygen species in adipocytes (Chen B).

HIF-1 regulation of adiponectin was examined by Natarajan Ramesh et al by isolating and characterizing the murine adiponectin promoter. HIF-1-dependent activation of the murine adiponectin promoter was verified via electrophoretic mobility shift assays, transient transfection assays, and QPCR. They showed for the first time that HIF-1 activation via an siRNA-mediated prolyl 4-hydroxylase-2 gene silencing strategy induced adiponectin mRNA expression in murine microvascular endothelium in vitro (17-fold), intact hearts and white adipose tissue. HIF-1α induced adiponectin expression was associated with improved myocardial viability in obese/diabetic mice and preservation of left ventricular function. This study suggest that local production of adiponectin by cardiomyocytes/microvascular endothelial cells may regulate cardiac function and indicate a novel strategy for protecting diabetic hearts from ischemia/reperfusion injury (Natarajan R).

Specific interaction of HIF-2a, but not HIF-1a, with the NF-kB Essential Modulator (NEMO) using immunoprecipitation, mammalian two-hybrid, and in vitro protein interaction assays was observed by Bracken et al (Bracken CP). In this context it
may be remembered that the immunomodulatory effect of adiponectin has been suggested to be mediated through a cross-talk between cAMP-PKA and NF-kB signaling (Ouchi N 2000).

Adiponectin can function to stimulate angiogenesis in response to ischemic stress by promoting AMP-activated kinase signaling. Therefore, adiponectin may be useful in the treatment for obesity-related vascular deficiency diseases (Shibata R). In adiponectin deficient mice endothelial NO (eNO) adiponectin deficiency drastically reduced levels of eNO in the vascular wall. Globular domain of adiponectin protects the vasculature in vivo via increased NO bioavailability with suppression of leukocyte-endothelium interactions (Ouedraogo R). There is evidence that loss of adiponectin induces a primary state of endothelial dysfunction with increased leukocyte-endothelium adhesiveness. Adiponectin could promote endothelial differentiation from peripheral blood CD14+ monocytes by morphology change, upregulation of endothelial cell (EC) markers and downregulation of smooth muscle cell markers. Adiponectin-promoted EC differentiation may contribute to vascular healing and angiogenesis (Yang H). Adiponectin protects against ischemia reperfusion induced myocardial damage following through AMPK and COX2 dependent mechanisms. Adiponectin induces cyclooxygenase-2 dependent production of prostaglandin E2 in cardiac cells and COX2 inhibition reversed inhibitory effect of adiponectin on TNF-α production and extent of myocardial damage (Shibata R). Adiponectin's protective action on heart from ischemia/reperfusion injury is at least partly by inhibition of iNOS and NADP-oxidase expression and resultant oxidative/nitrative stress (Tao L). Adiponectin strongly suppressed human aortic smooth muscle cells (HASMC) proliferation and migration through direct binding with PDGF-BB and generally inhibited growth factor–stimulated ERK signal in HASMC, suggesting that adiponectin acts as a modulator for vascular remodeling (Arita Y).
DIABETES AND METABOLIC SYNDROME

Diabetes mellitus is a heterogeneous group of metabolic disorders characterized by chronic hyperglycemia. Some forms of diabetes mellitus are characterized in terms of their specific etiology or pathogenesis, but the underlying etiology of the most common forms remains unclear but it is clear that a number of genetic defects can result in Diabetes mellitus.

The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. The prevalence of diabetes is higher in men than women, but there are more women with diabetes than men. The urban population in developing countries is projected to double between 2000 and 2030. The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people 65 years of age. The greatest absolute increase in the number of people with diabetes will be in India. It would home an estimated 79.4 million diabetic by 2030 (Wild S).

Diabetes mellitus is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. When fully expressed, diabetes is characterized by fasting hyperglycemia, but the disease can also be recognized during less overt stages, most usually by the presence of glucose intolerance. The effects of diabetes mellitus include long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, heart, and blood vessels. Diabetes may present with characteristic symptoms such as thirst, polyuria, blurring of vision, weight loss, and polyphagia, and in its most severe forms, with ketoacidosis or nonketotic hyperosmolarity, which, in the absence of effective treatment, leads to stupor, coma, and death. Often symptoms are not severe or may even be absent. Hyperglycemia sufficient to cause pathologic functional changes may quite often be present for a long time before the diagnosis is made. Consequently, diabetes often is discovered because of abnormal results from a routine blood or urine glucose test or because of the presence of a complication (Kahn CR).

Type 1 diabetes is usually an autoimmune disease primarily due to β-cell destruction. This usually leads to a type of diabetes in which insulin is required for
survival. Type 1 diabetes is less common compared to type 2 and will not be discussed here further. Type 2 diabetes is the most common form of diabetes. It is characterized by disorders of insulin action and insulin secretion, either of which may be the predominant feature. Usually, both are present at the time diabetes becomes clinically manifested. Although the specific etiology of this form of diabetes is not known, autoimmune destruction of the β-cells does not occur. Patients with type 2 diabetes usually have insulin resistance (i.e., resistance to insulin-stimulated glucose uptake) and relative, rather than absolute, insulin deficiency. At the time of diagnosis of diabetes, and often throughout their lifetimes, these patients do not need insulin treatment to survive, although ultimately many require it for glycemic control. This form of diabetes is associated with progressive β-cell failure with increasing duration of diabetes. Ketoacidosis seldom occurs spontaneously but can arise with stress associated with another illness such as infection (Kahn CR).

Most patients with type 2 diabetes (DMT2) are obese when they develop diabetes, and obesity aggravates the insulin resistance. The risk of developing type 2 diabetes increases with age, central obesity, and physical inactivity. Diabetes is also associated with increased circulating triglycerides, LDL, decreased HDL, hypertension, fatty liver, microalbuminuria, increased fibrinogen, increased plasminogen activator inhibitor-1, elevated plasma uric acid increased sympathetic neural activity. These risk factors are collectively named as metabolic syndrome (MS) and they seem to cluster quite often but not always in certain individuals and to certain extend genetically determined. People with DMT2 has an increased risk of nonalcoholic fatty liver disease (NAFLD), coronary artery disease (CAD), cardiovascular accidents (CVA), nephropathy and renal failure, neuropathy, fungal infections, chronic ulcers, cancer, cataract and retinopathy. Thus MS rather than a disease, is a tool in the hands of a physician/health care worker to identify people who are at an increased risk of having the above disorders [(Kahn CR) (Kahn R)].
INSULIN RESISTANCE

Insulin resistance is said to exist any time a normal amount of insulin produces a less than normal biologic response. Patients with insulin resistance have hyperinsulinaemia together with normoglycaemia or hyperglycaemia. Insulin resistance is commonly associated with obesity, non-insulin dependent diabetes mellitus, dyslipidaemia, essential hypertension, polycystic ovarian disease and a variety of genetic syndromes and in physiologic conditions such as puberty and pregnancy. Drugs such as corticosteroids, β blockers, and high dose thiazides can exacerbate insulin resistance; angiotensin converting enzyme inhibitors and (alpha) blockers may reduce resistance. Insulin resistance also is present in many states of stress, in association with infection, and secondary to treatment with a variety of drugs, particularly glucocorticoids. Reducing insulin resistance is important in managing type 2 diabetes for example, by losing weight, aerobic exercise, and stopping smoking; moderate alcohol consumption improves insulin resistance. Metformin, sulfonylureas, thiazolidinediones (PPAR-γ agonists) improves multiple aspects of the insulin resistance syndrome (Krentz AJ).

Insulin resistance can be further divided into states in which there is a rightward shift in the dose response to the hormone but the maximal response remains normal (decreased insulin sensitivity) or states in which the dose response is normal but the maximal response is decreased (decreased responsiveness), or a combination of the two (Kahn CR).

Insulin is the principal hormone controlling blood glucose concentration. Insulin initiates its action by binding to receptors in target tissues. Insulin receptor has 2 α subunits and 2 β subunits, linked by disulfide bonds. β subunit has intrinsic tyrosine kinase activity. Insulin binding to its receptor stimulates tyrosine kinase activity, which leads to tyrosine phosphorylation and recruitment of intracellular signaling molecules like IRS. A number of docking proteins bind to these cellular proteins and initiate the metabolic actions of insulin (GrB-2, She, Cbl, CAP, APS, SOS, SHD-2, p65, p110 and PI-3-kinase). For example activation of PI-3-kinase pathway stimulates translocation of GLUT-4 to cell surface which is crucial for glucose uptake in skeletal muscles and fat. Activation of other insulin signaling pathways
(Figure 5) induces glycogen synthesis, protein synthesis, lipogenesis and regulation of various genes in insulin responsive cells (Kahn CR).

Figure-5
Insulin Receptor Signaling

Source: Kahn CR, Weir GC, King GL et al. Joslin's Diabetes Mellitus. 14th Ed. Lippincott Williams and Wilkins

Muscle specific insulin receptor knock out (MIRKO) mice appeared normal, were able to maintain euglycemia up to at least the age of 20 months, glucose concentrations and insulin tolerance tests were indistinguishable between animals of knock out and control group. But there was a threefold increase of insulin-stimulated glucose transport in adipose tissue in MIRKO mice. Thus, insulin resistance in muscle produced a shift of substrate to adipose tissue that resulted in increased adipose-tissue mass, hypertriglyceridemia, and a modest increase in FFAs, all features of the metabolic syndrome associated with insulin resistance.

The livers of liver specific insulin knock out mice (LIRKO) were about 50% of normal size, and the insulin-receptor content of the liver was reduced by more than 90%. At 2 months of age, LIRKO mice were hyperglycemic in the fed state as compared with controls and exhibited severely impaired glucose tolerance. Serum insulin levels in LIRKO mice were markedly elevated owing both to an increase in islet size and insulin secretion and to a decrease in insulin clearance. The failure of these mice to respond to insulin in terms of suppression of hepatic glucose output
indicates that in mice the direct effect of insulin on this pathway is more important than any indirect effects.

Despite the importance of insulin for suppression of lipolysis, adipose tissue insulin receptor knock out (AIRKO) mice have normal blood glucose levels, normal glucose tolerance tests, and normal levels of FFAs and glycerol. In addition, all measures of insulin resistance are within the range of normal controls, including intraperitoneal insulin tolerance tests and fasting and fed serum insulin concentrations.

The FIRKO mice do, however, demonstrate a ~50% decrease in fat mass by 6 months of age that persists throughout life. FIRKO mice are also resistant to becoming obese as they age and even after treatment with gold thioglucose, an agent that produces a hypothalamic lesion and induces hyperphagia. As a result, these mice are also protected from the development of age- and obesity-related glucose intolerance. Perhaps most interesting is the extended life span of FIRKO mice, indicating the importance of body fat, rather than diet, in longevity (Kahn CR).

(This review on Diabetes Mellitus, Insulin Resistance and Metabolic Syndrome is based on the following book: Kahn CR, Weir GC, King GL et al. Joslin's Diabetes Mellitus. 14th Ed. Lippincott Williams and Wilkins)

Figure 6

Phenotypes of the insulin receptor (IR) and insulin receptor substrate (IRS) knockout (KO) mice. IGF-1, insulin-like growth factor-1

| IRS-1 KO mice | IGF-1 resistance – Growth retardation  
| | Insulin resistance (muscle, fat)  
| | β-cell hyperplasia but dysfunction  
| IRS-2 KO mice | Insulin resistance (liver)  
| | Defect in β-cell and neuronal proliferation  
| | Type 2 diabetes  
| IRS-3 KO mice | Normal birth weight  
| | Normal glucose homeostasis  
| IRS-4 KO mice | Normal growth  
| | Very mild defect in glucose homeostasis  
| IR KO mice | Normal intrauterine growth  
| | Severe insulin resistance; die in 3–7 days in diabetic ketoacidosis  

Source: Kahn CR, Weir GC, King GL et al. Joslin's Diabetes Mellitus. 14th Ed. Lippincott Williams and Wilkins
NONALCOHOLIC FATTY LIVER DISEASE

Introduction
Although alcohol-induced liver steatosis was already described by Thomas Addison in 1845, it is appreciated only since 1962 that steatosis can also occur without the use of alcohol, so-called non-alcoholic steatosis. The term nonacoholic steatohepatitis (NASH) was coined in 1980 to describe “the pathological and clinical features of non-alcoholic disease of the liver associated with the pathological features most commonly seen in alcoholic liver disease itself. In fact, the finding that some obese individuals had a liver disease histologically indistinguishable from alcoholic liver disease itself had long been recognized.

The term refers to a spectrum ranging from steatosis to steatohepatitis (NASH) to cirrhosis in the absence of alcohol abuse. NAFLD represents a range of diseases which results in “hepatic steatosis” in the absence of alcohol intake not more than 20g/day.

Fatty acids (FA) in excess can produce hepatic injury and non-alcoholic fatty liver disease (NAFLD). The later is a common disease. It is estimated that ~25% of the adult American population have fatty liver. In 10% of the sufferers, accumulated fat undergoes slow oxidation (lipid per oxidation) resulting in free radical damage and inflammation of the liver (NASH) and cirrhosis. The oxidative stress produced by lipid peroxidation plays an important role in the progress of fatty liver to NASH and eventually to cirrhosis. NASH has been linked to diabetes, high blood pressure, cholesterol and obesity. NASH is now recognized as one of the commonest causes of cirrhosis. In fact a significant proportion of ‘cryptogenic cirrhosis’ (cirrhosis due to unknown or unidentifiable cause) represents ‘burnt out’ NASH.

The worldwide prevalence of obesity is increasing at an alarming rate, with major adverse consequences for human health. According to World Health Organization (WHO), about one fifth of the world population is over weight. The W.H.O. predicts a doubling in the incidence of diabetes in the next two decades fuelled by modern lifestyles and an increasing incidence of obesity. Type 2 diabetes mellitus which
accounts for over 90% of diabetes cases is a manifestation of a much broader underlying disorder, the metabolic syndrome which includes a cluster of cardiovascular risk factors that in addition to glucose intolerance, includes obesity, hyperinsulinemia, dyslipidemia, hypertension, hypercoagulability, microalbuminuria and NAFLD. In the west, 50-70% of NAFLD have associated metabolic syndrome but there is a paucity of literature from India. This is very important considering the fact that India is currently the “diabetic capital” of the world (Widel S).

NAFLD AS THE LIVER MANIFESTATION OF A GENERALISED DISORDER IN FAT STORAGE AND MOBILIZATION

Logically if fat has to get accumulated there are four ways:

1) Increased Inflow (increased availability of lipids and lipid precursors)
2) Decreased Out flow/Secretion
3) Increased synthesis
4) Decreased oxidation

Increased Inflow

It has been observed that nearly 80% of people who undergo liver transplantation following cirrhosis from NASH get back the disease in few years. This point to the fact that at least in majority of the patients the cause lays outside the liver. Adipose tissue functions as an energy reserve; where energy can be stored in the most concentrated form viz. lipids safely unlike other tissue where it could cause ‘lipid toxicity’. This way it also acts as an energy sink in times of plenty. Soon after feeding the blood would be over loaded with energy rich compounds and the adipose tissue has an important role in clearing these compounds, especially lipids which are potentially harmful. However in chronic over nutrition adipocytes may become overloaded and may no longer be able to take up circulating lipids and glucose. Insulin is a stimulator of lipoprotein lipase the enzyme which mediates extraction of fatty acids from circulating lipoproteins which are the carriers of lipids as triglycerides (TG). Through the tissue-specific action of lipoprotein lipase, the TG-derived free fatty acids (FFA) are taken-up by peripheral tissues. Similarly the type-4 glucose transporters on adipocyte surface are also insulin dependent. Probably the
over loaded adipocytes may adopt insulin resistance (IR) as a strategy to save themselves from further overloading, damage and cell death (apoptosis?). As soon as the energy sinking capacity of the adipose tissue is exceeded the energy rich substrates starts 'over flowing' to other tissues like liver and muscle. This could be only one but the commonest mechanism of fatty liver. Nearly 60% of TGs deposited in liver in NAFLD comes from circulating nonesterified fatty acids. The TGs contained within adipose tissue are continuously being hydrolyzed into fatty acids and glycerol by the enzyme hormone-sensitive lipase (HSL). Insulin resistance impairs uptake of glucose from blood into skeletal muscle and adipose tissue; serum non-esterified fatty acid (NEFA) levels may also be elevated due to the failure of insulin to suppress HSL mediated lipolysis.

However (i) other mechanisms which induces IR in adipose tissue or defects in the transporters of lipids into adipose tissue which limits influx of lipids, (ii) defects in the control mechanisms involved in the release of lipids from adipocytes, (iii) defects in normal growth and proliferation of adipose tissue, could result in plasma lipid pooling and ectopic fat deposition such as fatty liver. As in the case of adipocytes hepatic fat overload may lead to hepatic insulin resistance (HIR). Therefore it should be possible to reverse fatty liver by 'unloading' the extra fat. This is indeed true. For example surgical obesity management techniques like gastric banding based on restriction of the gastric reservoir produces early satiety and reducing oral intake induces significant weight loss by unloading of fat reserves reverses fatty liver. Exercise and diet restriction and consequent fat reduction still remains the mainstream therapy in NAFLD. Interestingly removal of excess fat by liposuction does improve insulin sensitivity. This is possibly because as adipocytes are removed, the buffering action provided by them on circulating energy rich substrates is decreased.

A small part of the fatty acids released by HSL as described before are re-esterified within adipocytes while most fatty acids overflow into the blood which exceeds the normal oxidative needs of the body. Liver is another immediate buffer that has been shown to have a high capacity for accumulating fat and redirecting or oxidizing it later depending on the energy homeostatic signals. Within the liver, these fatty acids are either oxidized or re-esterified into TGs and secreted into the blood bound to
VLDL. The fatty acids re-esterified by the liver into TG are almost exclusively from adipose tissue lipolysis. Gluconeogenesis in liver needs supply of fatty acids and glycerol released from adipocytes. Glycerol contributes 90% of the substrate for hepatic gluconeogenesis in prolonged fasting mice. In man about 20% of hepatic gluconeogenesis is mediated by glycerol. Gluconeogenesis needs a coordinated efflux of glycerol from adipocytes and influx into hepatocytes through adipocyte specific glycerol channel, designated aquaporin adipose (AQPap/7) and a liver-specific aquaglyceroporin was also identified and named AQP9. Discord between efflux and influx channels possibly play an important role in the pathogenesis of NAFLD and diabetes mellitus.

Over flow of fat to liver could be due to increased dietary intake. Even after a short term fat feeding liver fat increases three fold without increase in visceral or skeletal muscle fat. Indeed the adipose tissue fat is an indicator of liver fat. The intrahepatic lipids increase by 22 % for any 1 % increase in total adipose tissue, by 21 % for any 1 % increase in subcutaneous adipose tissue and by 104 % for 1 % increase in intra-abdominal adipose tissue. Thus liver bears the brunt as soon as adipose tissue buffering reaches its limit.

Zucker rats (fa/fa) have inactivating mutation in the leptin receptor and hence obese and develops fatty liver. Liver specific correction of leptin receptor deficiency results in reduced TG accumulation in the liver but not in other non-adipose tissues. This could be an example of adipokine mediated communication between adipose tissue and liver. Leptin in conditions of ‘calorie excess’ signals liver to increase lipid oxidation and to down regulate to lipid synthesis and thus protect it and other organs from steatosis. Ob/ob mice with mutations in Scd1 had histologically normal livers with significantly reduced triglyceride storage and VLDL production. Down regulation of SCD1 is an important component of leptin’s metabolic actions. As the buffering capacity of liver also exceeds its limit lipid starts getting accumulated in cardiovascular system and other organs. The macrophages associated with blood vessels, the evolutionary kins of adipocytes and hepatocytes, will try to “buffer” the excess lipids by phagocytosis and later oxidize them partly to form toxic free radicals and immunogenic compounds. They become ‘foamy cells’ as their capacity
to accumulate fat reaches the limit. This eventually leads to “fibrosis” of vessel wall (atherosclerosis homologous to cirrhosis of liver) and an increase in CVS related mortality. Indeed some studies published recently find the seeds of CVS mortality in NAFLD.

Defects in fatty acid transporters in adipocytes also could result in hypertriglyceridemia and subsequent increased inflow of FA to liver. For example CD36-/- mice lacking the fatty acid transporter that is normally present in muscle and adipose tissue showed increased hepatic TG content and a decreased sensitivity of hepatic glucose production to insulin.

There are multiple genetic defects which culminate in adipose tissue deficiency and adipose tissue deficiency invariably results in fatty liver. Lean transgenic mice that express ZIP/F-1 protein in adipose tissue, which blocks the function of several classes of transcription factors, are insulin resistant and developed fatty liver. Interestingly in these mice upon transplantation of fat tissue insulin resistance as well as the fatty liver disappeared. PPAR-γ mutant mice develop IR and fatty liver in conjunction with the terminal atrophy of adipose tissue. Sterol regulatory element-binding proteins (SREBPs) are a family of transcription factors that activate the entire program of cholesterol and fatty acid synthesis. The transgenic mice that overexpress nuclear SREBP-1c (aP2-SREBP-1c) exclusively in adipose tissue have very little adipose tissue, apparently as a result of disturbed adipocyte differentiation. They develop severe hyperglycemia, hyperinsulinemia, and lipid accumulation in liver. The fatty liver dystrophy (fld) mutant (lipin-deficient) mice are characterized by neonatal fatty liver and hypertriglyceridemia that resolve at weaning, glucose intolerance and increased susceptibility to atherosclerosis and neuropathy. Adult fld/fld mice exhibits 80% reduction in mass both white and brown fat pads compared with wild-type controls, and consist of immature adipocytes. Human examples of adipose tissue deficiency and consequent fatty liver is described else where in this paper.
Increased Intake into cells

The lipid intake is determined by lipoproteins which are carriers of lipids to liver predominantly from adipose tissue and digestive tract through the circulatory system. The lipids are further transported into hepatocytes by various 'lipid transporters' which are membrane bound channels and cellular lipid binding proteins like fatty acid transport proteins (FATP), fatty acid translocase (FAT/CD36), fatty acid binding proteins (FABP), caveolin-1 etc. Tissue accumulation of FA requires intracellular trapping involving association between many members of intracellular FA binding proteins.

FATP is highly expressed in hepatocytes and adipocytes that reveal high-level FA uptake for both metabolism and storage. FATP1 is found in adipose tissue and in the heart. FATP2 and FATP5 are expressed in the liver, while FATP4 is expressed in the intestine. Overexpression of FATP5 in cultured cells has been shown to increase FFA uptake while knock out of FATP-5, results in decreased accumulation of fat in liver and decreased production of conjugated bile acids. A recent study found an upregulation of FABP4 and FABP5 in NAFLD independent of obesity. Thus FATP5 is an important membrane protein involved in fatty acid accumulation by the liver. In mice which are FAT/CD36-deficient, the flux of fatty acids toward the liver is increased, precipitating steatosis, but there without any evidence of an increase in hepatic VLDL production.

The fatty acid taken up by liver is oxidized and excess is esterified and accumulated or secreted. Esterification is most efficient with mono-unsaturated lipids as monounsaturated fatty acyl-CoAs are the preferred substrates for the lipid synthesis of triacylglycerol (TAG) in the endoplasmic reticulum (ER). This is possibly one reason why loss of members of desaturation enzyme family, stearoyl-CoA desaturase which catalyses the rate-limiting step in the synthesis of monounsaturated fatty acids, particularly oleate (18:1) and palmitoleate (16:1), protects mice against fatty liver in mice.
Decreased outflow
The increased hepatic uptake and biosynthesis of FAs are compensated through increased removal of lipids from the liver. In this process, VLDL plays a central role. The principal apoprotein for this particle is apoB100, but apoE and apoC-I, C-II, and C-III are incorporated as well. Lipid homeostasis in mammalian cells is regulated by a family of membrane-bound transcription factors designated sterol regulatory element–binding proteins (SREBPs). In the liver, three SREBPs regulate the production of lipids for export into the plasma as lipoproteins and into the bile as micelles. nSREBP-1α transgenic mice develop a massive fatty liver engorged with both cholesterol and triglycerides.

The bile acid receptor farnesoid X receptor (FXR; NR1H4) is a central regulator of bile acid and lipid metabolism. FXR protects the liver from the deleterious effect of bile acid overloading by inhibiting their biosynthesis and stimulating their excretion. FXR regulates the expression of several apolipoproteins involved in the transport and metabolism of lipids.

Bile acid reduces the secretion of VLDL by repressing microsomal triglyceride transfer protein (MTTP) which mediates lipidation of apoB 100 to form VLDL. This effect is possibly mediated through FXR. Pharmacologic agents that induce hepatic steatosis like amiodarone, tetracycline, pirprofen, tianepine inhibited MTTP activity. However it is still unclear whether FXR mediated decrease in triglyceride rich VLDL secretion and consequent decrease in lipid out flow from liver is important in the pathogenesis of fatty liver.

Abetalipoproteinemia, a genetic disease which is associated with fatty liver is caused by mutations in the MTTP gene resulting in blockage of VLDL assembly and secretion. MTTP -493 G/T polymorphism may influence NASH by modulating postprandial lipemia and lipoprotein metabolism; homozygous GG carriers have a more atherogenic postprandial lipid profile than the other genotypes, independently of adipokines and insulin resistance. Fatty acid level is sensed in liver by PPAR-α and genes which promote lipid secretion like FABP, MTTP and apoB100 are upregulated. Indeed this could be one mechanism by which fibrates which are
PPAR-α agonists used in the treatment of NAFLD exerts its effect. Since lipid synthesis should go hand in hand with lipid secretion; as lipogenesis is increased by activation of the liver X receptor, hepatic VLDL production is also increased. Selective modulators of nuclear receptors involved in lipid homeostasis could thus revolutionize the treatment of NAFLD, Gall Stone disease, obesity and type2 diabetes mellitus.

HDL particles participate in reverse cholesterol transport, the mechanism by which cholesterol from extrahepatic tissues returns to the liver for excretion as biliary cholesterol. Low HDL-cholesterol is associated with metabolic syndrome and NAFLD. ABCA1 is a lipid binding protein which increases reverse cholesterol transport to pre-beta HDL. Patients with low HDL-cholesterol and abnormal cellular lipid efflux due to ABCA1 gene defects (Tangier disease) also have elevated plasma triglycerides and fatty liver.

**Increased synthesis**

The synthesis of lipid and lipoproteins is important to metabolic disorders. In NAFLD nearly a quarter of the accumulated fat comes from denovo lipid (DNL) synthesis and hepatic lipid synthesis is markedly increased in NAFLD. Acetyl coenzyme A (acetyl CoA) is a crucial metabolic intermediate in carbohydrate and protein catabolism towards lipid synthesis. Acetyl CoA carboxylase (ACC) and Fatty Acid synthetase are two major enzymes that drive DNL in the liver. Inhibitors of ACC decreases lipid accumulation by hepatocytes and might prove useful in the development of novel therapeutic agents to combat fatty liver.

Synthetic pathways for triacylglycerol (TAG), cholesterol and its ester, and phospholipid are separate, but transcriptionally co-regulated. In insulin sensitive tissues and particularly in the liver, the transcription factor SREBP-1c transduces the insulin signaling regulating lipid synthesis. Overexpression of nSREBP-1c in the liver of transgenic mice bypasses insulin requirement and activates the same genes stimulated by insulin and produces a triglyceride-enriched fatty liver with no increase in cholesterol. The mRNAs for fatty acid synthetic enzymes and rates of fatty acid synthesis are elevated fourfold in liver. Alcohol induces fatty liver partly
by impairing of PPAR-α and PPAR-γ activity and activation of SREBP-1. Overexpression of nSREBP-2 in liver increases the mRNAs encoding all cholesterol biosynthetic enzymes.

Under physiological conditions, SREBP-1c is transiently induced in the liver by insulin through activation of IRS-2; this causes a switch from glycogen synthesis to lipid synthesis. To complete a feedback loop, SREBP-1c then suppresses IRS-2 transcription. Under certain pathogenic conditions, expression of SREBP-1c in the liver remains elevated, and this increases lipid synthesis with resultant accumulation of fat.

Hepatitis B virus (HBV) infection is associated with fatty liver in a significant proportion of patients. HBV encoded protein HBx causes lipid accumulation in hepatic cells which is mediated through SREBP1 and PPAR-γ.

Obesity and insulin resistance is a pro-inflammatory state characterized by increased levels of pro-inflammatory cytokines. Cytokines like IL-6 and TNF-α further promotes insulin resistance by increasing hepatic suppressors of cytokine signaling (SOCS) expression. Over expression of SOCS-1 and SOCS-3 in liver causes insulin resistance and an increase in the key regulator of fatty acid synthesis in liver, SREBP-1c. In obesity, increased SOCS proteins enhance SREBP-1c expression by antagonizing STAT3-mediated inhibition of SREBP-1c promoter activity. Interestingly, n-3 PUFAs downregulate SREBP 1-c, which increases transcription of genes responsible for fatty acid synthesis such as fatty acid synthase and stearoyl Co-A desaturase.

The liver has a central role in glucose homeostasis. On feeding, glucose influx triggers gene expression changes in hepatocytes to suppress endogenous glucose production and convert excess glucose into glycogen or fatty acids to be stored in adipose tissue. This process is controlled by insulin, although debate exists as to whether insulin acts directly or indirectly on the liver. Transcriptional activation of glycolytic and lipogenic genes requires the presence of both insulin and glucose, neither of which is active alone. Recently, carbohydrate responsive element binding
protein (ChREBP) emerged as a pivotal transcription factor implicated in the regulation of lipogenic genes by glucose.

Liver xenobiotic receptor (LXR) is another glucose sensor which is activated by glucose and switches on several genes involved in fatty acid synthesis. An LXR-binding site in the SREBP-1c promoter activates SREBP-1c transcription in the presence of LXR agonists. When lipogenesis is increased by pharmacological activation of the liver X receptor, hepatic VLDL production is increased 2.5-fold, and the liver produces large TG-rich VLDL particles. Interestingly, glucose induces expression of LXR target genes involved in cholesterol homeostasis like ABCA1 which is defective in Tangier disease.

A common feature of many metabolic pathways is their control by retinoid xenobiotic receptor (RXR) heterodimers. It is interesting to note that LXR heterodimerises with RXR. Promiscuous RXR also heterodimerises with PPAR members. PPAR-α plays a pivotal role in fatty acid catabolism in liver by upregulating the expression of numerous genes involved in mitochondrial fatty acid oxidation. Thus RXR is a common partner of two nuclear receptors acting in opposite directions with regard to fatty acid metabolism. So both LXR and PPAR-α compete for the limited pool of RXR and this dynamic equilibrium determines the direction of lipid metabolism.

FXR also plays a key regulatory role in glucose homeostasis. FXR-null mice developed severe fatty liver and elevated circulating FFAs, which was associated with elevated serum glucose and impaired glucose and insulin tolerance. Activation of FXR lowers plasma glucose levels in fasted, fed, diabetic mice. Bile acids, by activating FXR, induce the expression of short heterodimer partner (SHP). SHP then interferes with SREBP-1c expression by inhibiting the activity of LXR and eventually other transcription factors that stimulate SREBP-1c expression (Sanal MG).

An increase in serum triglyceride concentrations and fatty liver have been observed in patients with malabsorption of bile acid as found in chronic inflammatory bowel
disease, ileal resection and cholestyramine treatment. This could be due to loss of bile salt mediated inhibitory effect on fatty acid synthesis mediated through FXR and further research is required in this area.

It may be noted that NAFLD is classically associated with gall stone disease and hypertriglyceridemia. The increased incidence of gall stones at least partly due to decreased secretion of bile salts which is a potent emulsifier and the consequent instability of bile pigments resulting in precipitation and stone formation. It would be logical to hypothesize that defective FXR expression or signaling and consequent deficiency in bile inhibition of fatty acid synthesis might play a role in certain cases of hepatic steatosis associated with biliary stones (Sanal MG).

Hepatocyte Nuclear Factor -4α (HNF-4α) is a transcription factor which is mutated in monogenic autosomal dominant non-insulin-dependent diabetes mellitus type I (MODY-1), controls the expression of several genes, including hepatocyte nuclear factor 1α (HNF-1α), a transcription factor which regulates the expression of several hepatic genes and the human CYP7A1 gene in bile acid synthesis and phosphoenolpyruvate carboxykinase (PEPCK) gene in gluconeogenesis. Long-chain fatty acids, including palmitic acid, have been identified as endogenous HNF-4α ligands and this allows the transcriptional control of gluconeogenesis during active lipid synthesis. It is interesting to note that glucose and fructose induces lipogenesis, reduces hepatic HNF-4α levels, which in turn attenuates the expression of sex hormone binding globulin (SHBG), a biomarker of metabolic syndrome. Thus HNF-4α is another major protein at the cross roads of sex hormones, diabetes, fatty liver, dyslipidemia and gall stone disease.

Nuclear receptors are notoriously promiscuous. They are known to be activated, inhibited or otherwise modulated by numerous xenobiotic compounds which include alcohol, drugs, insecticides, dietary contaminants and numerous other chemicals acquired from environment. This could explain the association of certain drugs as well as environmental toxins with fatty liver. It may be noted that many of the denovo lipid synthesis pathways described above are shared by both alcoholic and nonalcoholic fatty liver disease.
Fatty acid oxidation is compartmentalized in eukaryotic organisms into three subcellular organelles, with beta oxidation confined to mitochondria and peroxisomes and CYP4A-catalyzed omega oxidation occurring in the endoplasmic reticulum. Beta oxidation is primarily involved in the oxidation of fatty acids with carbon chains not longer than twenty while peroxisomal oxidization predominantly deals relatively complex and toxic fatty acids containing twenty or more carbon atoms and C27-bile acid intermediates. Microsomal omega oxidation system hydroxylates saturated and unsaturated fatty acids which will eventually be oxidized by the other two oxidation systems. Some of the key enzymes of these three fatty acid oxidation systems in liver are regulated by PPAR-α. When mice fed on a methionine-choline deficient (MCD) diet—a dietary model of fibrosing steatohepatitis—were treated with the PPAR-α agonist, Wy-14,643, developed significantly less steatohepatitis, markedly lower ALT levels and less lipid peroxidation compared to controls. This occurred despite a marked increase of the liver P-450 enzymes which are oxidant stress inducers. Mice lacking PPAR-α (PPARα/-) fail to respond to the inductive effects of peroxisome proliferators, whereas those lacking fatty acyl-CoA oxidase (AOX/-), the first enzyme of the peroxisomal beta-oxidation system, exhibit extensive microvesicular steatohepatitis, leading to hepatocellular regeneration and massive peroxisome proliferation, implying sustained activation of PPAR-α by natural ligands. Ethanol a common cause of fatty liver interferes with DNA binding and transcription-activating properties of PPAR-α, as demonstrated with cultured cells and in ethanol-fed mice. Treatment of ethanol-fed mice with a PPAR-α agonist can reverse fatty liver even in the face of continued ethanol consumption.

Mitochondrial beta oxidation results in shortening of fatty acids and FA are oxidized carbon by carbon into acetyl-CoA subunits, which either condenses into ketone bodies that serve as oxidizable energy substrates for extrahepatic tissues, especially during starvation, or enter into the tricarboxylic acid cycle. Mitochondrial beta oxidation is regulated by carnitine palmitoyl transferase-1 (CPT-1), the carnitine concentration and malonyl-CoA, which inhibits CPT1. Fatty acids, fatty acyl-CoAs and several structurally different chemicals known as peroxisome proliferators, which activate PPAR-α, regulate CPT1 levels in the liver. Inhibition of fatty acid
oxidation in the liver is an intrahepatic cause of the development of liver steatosis. For instance, etomoxir, a CPT-1 inhibitor, inhibits fatty acid oxidation and induces steatosis.

Thioredoxin (TRX) is an important redox regulator protein that has a redox-active dithiol/disulfide in its active site. TRX binding protein-2 (TBP-2) was identified as a TRX binding protein from a yeast two-hybrid system study, and as a negative regulator of TRX through direct interaction. TBP-2 null mice are viable and fertile, but under fasting conditions, their survival rate was sharply reduced, concomitant with fatty liver, hypoglycemia, dyslipidemia, severe bleeding and hepatic and renal dysfunction. This mouse has been proposed as an animal model for Reye's syndrome an acute illness which is encountered exclusively in children below 15 years of age characterized clinically by vomiting and signs of progressive central nervous system damage, hypoglycemia and extensive fatty liver and liver damage. TBP-2 is also reported to be a causative gene for familial combined hyperlipidemia (FCHL) in mice. Individuals with genetic deficiencies in fatty acid transport and mitochondrial oxidation show a similar pathology to Reye syndrome, which is defined as a Reye-like syndrome, such as CPT-1 deficiency. Defects in different enzymes involved in fatty acid utilization give rise to different disorders. A defect in long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) is associated with acute fatty liver of pregnancy (AFLP) while medium-chain acyl-CoA dehydrogenase (MCAD) defect is involved in sudden infant death syndrome (SIDS).

Less is understood about the role of mitochondrial beta oxidation in development of NAFLD. Mitochondrial trifunctional protein (MTP) catalyzes long-chain fatty acid oxidation. Chronic alcoholism is known to cause mitochondrial damage. Megamitochondrion with crystalline inclusions is a feature of NAFLD underscoring its importance in pathogenesis. A mouse model for MTP deficiency reported that homozygous (MTPa-/-) mice suffer neonatal death. Heterozygosity for fatty acid oxidation defects predisposes to NAFLD and insulin resistance in aging mice. Thus impaired mitochondrial oxidation may play an important role in pathogenesis of NAFLD. Primary or age-related defects in mitochondrial beta oxidation could play a role in development of NAFLD. MTP defects in man are recessively inherited and children with defects of any of the three enzymatic functions exhibit mostly
microvesicular hepatic steatosis. A diet that provides 2–5% of energy from (n-3) and (n-6) PUFA leads to a coordinate suppression of glycolytic and lipogenic genes and to an induction of genes involved in fatty acid oxidation. This metabolic balance in liver leads to a 'partitioning' of fatty acids away from triglyceride synthesis toward fatty acid oxidation. Long-chain n-3 PUFAs upregulate PPAR-α, increases transcription of genes responsible for fatty acid oxidation, such as mitochondrial CPT 1 and peroxisomal acyl-CoA oxidase. Thus PUFA intake could be beneficial in treatment of NAFLD.

EPIGENETIC FACTORS

The thrifty phenotype hypothesis (Barker's hypothesis) postulates epigenetic memory of the fetal/neonatal environment. Feeding rats a high-carbohydrate diet during suckling rapidly leads to hyperinsulinism. Moreover, when these female rats become pregnant, hyperinsulinism also rapidly appears in their offspring even though they are fed a normal diet, suckling milk from their mothers. Epigenetic regulation of gene expression involves chemical modification of chromatin by enzymes such as sirtuins (SIR2-related proteins), whose activities are linked to cellular energy stores and in lower organisms, sirtuin signaling interface with insulin signaling pathways and Sir2 orthologues could possibly be involved in the pathogenesis of fatty liver disease.

TIMERS OF FATTY LIVER DISEASE

Many biological processes are timed and there is a biological clock which keeps the time at the molecular level. Recent studies have revealed that endogenous rhythms are generated at the cellular level by circadian core oscillators, which are composed of transcriptional/translational feedback loops involving a set of CLOCK genes. Obese diabetes attenuated this rhythmic expression in most of the clock and adipocytokine genes in adipose tissue. There was impairment in the rhythmic expression of the CLOCK genes in the liver as well. Interestingly, pioglitazone treatment improved the attenuated rhythmicity in the liver, but not the adipose tissue. A recent human study suggests a potential role of the CLOCK gene variants and their related haplotypes increased susceptibility to NAFLD and disease progression.
The Classic ‘Metabolic Syndrome Associated NAFLD’

The ‘classical NAFLD’ which is the ‘fatty liver of affluence’ is the commonest form and is associated with metabolic syndrome and can be viewed as a special class of adipose tissue malfunction and disordered lipid homeostasis. MS can be considered as inappropriate expression of ‘thrifty genes’ resulting in obesity, abnormal fat deposition, dyslipidemia, diabetes, pro-inflammatory (and pro-neoplastic?) states. NAFLD, which is characterized by abnormal fat deposition in liver and chronic pro-inflammatory state, has been considered as a liver manifestation of MS. This is substantiated by the epidemiological data where 50-80% of the NAFLD has associated MS depending on the area studied and methods adopted. The association of NAFLD with MS and IR has been extensively reviewed by Marchesini et al. and Cortez-Pinto et al.

Metabolic Syndrome is a tool for identifying individuals who are at the cardiovascular accidents rather than a single disease. NAFLD is gaining importance in this context. Recent studies have reported the association of NAFLD with multiple classical and non-classical risk factors for cardiovascular disease (CVD). Moreover, there is a strong association between the severity of liver histopathology in NAFLD patients and greater carotid artery intima-media thickness and plaque, and lower endothelial flow-mediated vasodilation (as markers of subclinical atherosclerosis) independent of obesity and other MS components.

Identification of MS is very useful not only as a tool to identify people with cardiovascular and diabetic risk but also NAFLD but much confusion exists in the criteria of MS. Criteria for MS should be convenient but flexible to the regional and ethnic differences is important in establishing its association with NAFLD, a disease which progress asymptomatically to cirrhosis. There exists significant genetic environmental and life style diversity in population across different continents and cultures. For example Asian Indians are prone to central obesity and for same degree of weight gain are more insulin resistant.

In case of other well known syndromes, a single or a few well defined genetic defects results in the clustering of a host of strongly associated phenotypic features.
But in MS, a host of poorly defined genetic defects results in loosely associated clustering of phenotypic features. The primary underlying causes of the metabolic syndrome are thought to be insulin resistance. Central obesity almost certainly is a major cause of insulin resistance. There is considerable doubt whether all patients with the metabolic syndrome are indeed insulin resistant. Many nondiabetic adults with a wide range of age and body mass are hyperinsulinemic and insulin resistant (~50%), ~25% are insulin resistant but without hyperinsulinemia and the same proportion are hyperinsulinemic but without insulin resistance. Thus the lack of consensus regarding MS criteria, insulin resistance cut off value together with the complex pathogenesis of hepatic steatosis makes it difficult to evaluate their association.

The Lean Nonalcoholic Fatty Liver Disease

It became clear that Non-alcoholic steatohepatitis (NASH) has an equal sex distribution and that many, perhaps even the majority of patients according to some reports are neither obese nor diabetic.

We already described the importance of adipose tissue in fatty acid buffering. Several congenital forms of lipodystrophy are associated with fatty liver. Berardinelli-Seip syndrome caused by mutation of BSCL2 gene is characterized by a near-total lack of body fat from birth. Interestingly BSCL2 gene is highly expressed in the brain but only modestly in adipocytes, suggesting a role for the central nervous system in the pathogenesis. MRI studies also reveal a near-complete absence of metabolically active adipose tissue from most subcutaneous areas, intraabdominal and intrathoracic regions and bone marrow. Fatty liver has been noted during infancy and can lead to cirrhosis and its complications. They show extreme insulin resistance. Dunnigan variety of familial partial lipodystrophy and Mandibuloacral dysplasia (type-A lipodystrophy) are associated with laminin gene mutation causes fatty liver and IR. PPAR-γ mutations result in a dominant form of lipodystrophy and fatty liver. Similarly in acquired lipodystrophies like Lawrence syndrome; hepatomegaly due to fat infiltration is a consistent finding.

In patients who undergo liposuction or other forms of obesity reduction surgery, fatty liver is a common finding. Highly active antiretroviral therapy (HAART) therapy for HIV, a combination which includes HIV-1 protease inhibitors, is
associated with the development of lipodystrophy in the majority of patients after 18 months to 2 years of treatment. It is characterized by fatty liver following marked reduction in subcutaneous fat from the face, trunk and limbs, resulting in an appearance of "increased muscularity". These patients are prone to develop insulin resistance, hypertriglyceridemia and fatty liver. In animal models of lipodystrophy IR and fatty liver are corrected by infusion of adipokines. This underscores the role of adipokines in modulating the response of adipose tissue and as messengers to other organs in response to changing energy environment and adaptive mechanisms like IR. Removal of adipose tissue causes IR and fatty liver in hamsters. It is well known that patients who undergo liposuction as a treatment for obesity do not show metabolic benefits. It won't be surprising if liposuction worsens insulin resistance and fatty liver as this procedure removes active adipocytes from subcutaneous spaces. Thus, any defect that prevents adipose tissue from acting as an 'energy rich substrate sink' would result in fatty liver.

The Fatty Liver Associated with Infectious and Immunological Disorders

Fatty liver is associated with many chronic inflammatory conditions. Hepatitis C especially type 3 genotype is associated with fatty liver in nearly 50% of the patients. In patients with chronic liver disease due to hepatitis C virus, the major adipokine adiponectin was positively correlated with hepatic inflammation and adiponectin receptors were differentially regulated in the setting of hepatic insulin resistance. It has been shown, that HCV internalization is facilitated via LDL (low density lipoprotein) receptors and the virus enters into the cell via endocytosis. More recently, a broadly expressed lipoprotein binding receptor, the human scavenger receptor class B type I was shown to serve as a receptor for HCV. According to Younossi the HCV genotype 3 core protein inhibits very low-density lipoprotein (VLDL) secretion, leading to hepatic steatosis. Several studies demonstrate that steatosis disappears after patients with hepatitis C genotype 3 achieve a sustained response. The resolution of steatosis after treatment strongly supports the association between hepatic steatosis and HCV genotype 3.

Though less frequently, Hepatitis B is also associated with fatty liver. The hepatitis B virus encoded protein, HBx causes lipid accumulation in hepatic cells which mediated is by SREBP1 and PPARγ. Fatty liver has been reported with certain
bacterial infections like Q fever. Autoimmune diseases like systemic lupus erythematosis (SLE) and rheumatoid arthritis etc are also associated with fatty liver.

There are reports that NAFLD is characterized by a low-grade systemic inflammation. This could be partly because of the association of NAFLD with obesity which is a pro-inflammatory state. Conditions that induce inflammatory state in liver might in long term stimulate adipocytes and macrophages because they have a common ancestry and hence they share many cytokines, growth factors and signaling pathways. There are many ways by which fat is deposited in liver but it is the body’s response to fat and its derivatives which determines the progress of the disease from simple fatty liver to steatohepatitis and cirrhosis.

The Xenobiotic Fatty Liver
NASH features are also encountered as an adverse reaction to a few drugs—for example, amiodarone and perhexilene. Drug like methotrexate, aspirin, vitamin A, glucocorticoids, amiodarone and synthetic estrogen causes macrovescicular steatosis while microvescicular is caused by valproic acid, tetracycline, nucleoside analogues etc. Chronic alcoholism causes predominantly macrovescicular steatosis. However some hepatocytes in these livers with microvescicular steatosis may also reveal a macrovesicular fatty change, implying that with the progression of the disease some of these small lipid vacuoles may fuse to become a large droplet.

The metabolism of ethanol enhances the level of NADH in the liver which, in turn, stimulates the synthesis of fatty acids and their incorporation into triglycerides. Ethanol mediated impairment or inhibition of PPAR-α and PPAR-γ and stimulation of SREBP, the receptor molecules that control the enzymes responsible for the oxidation and synthesis of fatty acids, respectively, appear to contribute to the overall lipid load in the alcoholic liver.

These xenobiotics cause steatosis by a host of mechanisms. Drugs like tetracycline, aspirin etc do so predominantly by inhibiting hepatic fatty acid oxidation. Jamaican vomiting syndrome which is caused by hypoglycin-A present in unripe ackee fruit is an example for natural toxins inducing fatty liver. Estrogen, various pesticides etc probably induces various nuclear receptors like FXR, PXR, CXR, LXR, PPARs etc
involved in lipid homeostasis as these nuclear receptors are promiscuous. There is a paucity of literature and much research has to be done in this field.

**Fatty Liver Associated with Defective Fatty Acid Catabolism**

Reye's Syndrome is an acute illness encountered exclusively in children below 15 years of age classically following a viral infection and the use of aspirin. It is characterized clinically by vomiting and signs of progressive central nervous system damage, signs of hepatic injury and hypoglycemia. Morphologically, there is extensive fatty vacuolization of the liver and renal tubules. There is mitochondrial dysfunction with decreased activity of hepatic mitochondrial enzymes. There are reports linking Thioredoxin Binding Protein-2, a protein that interacts with thioredoxin which is an important redox regulator protein and Rye's syndrome. Individuals with genetic deficiencies in fatty acid transport and mitochondrial oxidation show a similar pathology to Reye syndrome, which is defined as a Reye-like syndrome, such as CPT deficiency.

Acute fatty liver of pregnancy (AFLP) is a syndrome that occurs late in pregnancy and is often associated with jaundice and hepatic failure. AFLP has an incidence of 1 per 13,000 deliveries. It affects women of all ages and races. The liver is typically small. AFLP is more common when the mother is carrying a male fetus and associated with a deficiency of long-chain-3-hydroxy acyl COA dehydrogenase (LCHAD). Preeclampsia or the HELLP syndrome, which may complicate eclampsia, presents in a similar fashion and progresses to severe liver dysfunction, though typically with a normal size liver. Aminotransferase elevations are typically modest in all of these conditions.

An association between recurrent maternal acute fatty liver of pregnancy with a fetal fatty acid oxidation disorder was reported first in two siblings who both died at 6 months of age. In another study involving 11 pregnancies in 5 mothers where 6 babies had confirmed deficiency of LCHAD, by enzymatic analysis of cultured skin fibroblasts. The mothers had either AFLP or HELLP syndrome in all six pregnancies with the LCHAD deficient fetuses. However how fetal defects induce acute fatty liver in mothers is still not clear.
Fatty Liver in Malnutrition

Protein malnutrition, especially in infancy and early childhood, accounts for most cases of severe fatty liver in the tropical zones of Africa, South America, and Asia. The hepatic changes may be associated with other clinical and pathologic features of Kwashiorkor. High plasma levels of free fatty acids are generally regarded as a key biochemical feature of the condition. Glucose intolerance is another characteristic feature. Interestingly in marasmus, a form of protein-calorie malnutrition, fatty liver is not part of the clinical picture.

The Benign Fatty Liver-The Non-progressive Fatty Liver

Kagansky et al. evaluated 91 octogenarians (mean age, 85.56 ± 3.76 years) to determine the prevalence and the clinical presentation of NAFLD in the elderly. Among these patients, who had been admitted to a geriatric hospital, about 50% were diagnosed with NAFLD. An association of NAFLD with the metabolic syndrome or advanced liver disease was not observed in this older patient population. These data indicate that NAFLD is a common and relatively benign finding in the elderly. Another study, examined sequential liver biopsies obtained from 103 NAFLD patients. The mean interval between biopsies was about 3 years and found that the stage of fibrosis slowly progressed in 37% of patients but remained stable in 34%. The observation that NAFLD patients without NASH have a benign prognosis is confirmed in several other studies as well.

It is possible that fatty liver is physiological than pathological under certain circumstances for example after a fat-carbohydrate rich diet for a short period. Increase in hepatic TG content can occur within 10 days after starting the high-fat diet in mice. Overnight fasting increases plasma FFA to such an extent that liver TG content increases in dogs. It is logical to assume that as in laboratory animals transient fatty liver follows a short term calorie rich diet in man as well. Unfortunately there is no diet related guide line for ultrasonological determination for fatty liver in the diagnosis of NAFLD. Ideally the subject should be on 'normal' and balanced diet at least for a week prior to ultrasonological evaluation for NAFLD. In a large population based study involving 4401 subjects, it was observed that fatty liver regressed in a significant number of participants; fatty liver regressed in 14% of men and 25% of women. In another follow up study involving sequential
In another recent study fatty liver regressed in nearly 1 of every 2 cases and had a substantially benign course. A carbohydrate rich but fat deficient diet may predispose to fatty liver as surplus carbohydrate induce adipogenic genes. This could well explain the fatty liver which follows total parenteral nutrition (Sanal MG).

(This review on NAFLD is based on the following: Sanal MG. The blind men 'see' the elephant-the many faces of fatty liver disease. World J Gastroenterol. 2008; 14(6):831-44)
Plate 1

Upper: The photomicrograph shows several of the components that comprise the constellation of features to be evaluated for grading. Steatosis is predominantly macrovesicular, ballooned hepatocytes are noted, and some contain intracytoplasmic material consistent with poorly formed Mallory's hyaline. Mild, acute, and chronic inflammation are present (hematoxylin and eosin, 20x).

Lower: Photomicrograph of cells with easily identified Mallory bodies (small arrows).

Upper: Zone 3 field, the pericellular fibrosis is noted predominantly around the ballooned hepatocytes (40x). The Masson’s trichrome stain for collagen highlights perivenular and zone 3 perisinusoidal fibrosis, which is characteristic of the fibrosis in steatohepatitis.

Lower: Photomicrograph of cells with easily identified Megamitochondria (big arrows) H&E x400.

Fatty liver: gross appearance, autopsy specimen. Note the change of color from normal brown to yellowish brown and the ‘oily’ appearance.
Left: A sonogram of a fatty liver showing increased echotexture compared with the adjacent kidney (bright liver). This is not a specific finding for NAFLD. Right: Geographic fatty liver MRI image (RadioGraphics 2006; 26:1409-1418)

Left: Steatosis and Fibrosis following amiodarone therapy, an unenhanced computed tomography (CT) scan of the upper abdomen. The liver has a greatly increased density (111.8 HU). Source: World J Gastroenterol 2005;11(34):5394-5397. Right: Severe fatty liver in lipodystrophy (MRI image): lamin A and C mutation. The Journal of Clinical Endocrinology & Metabolism 88(3):1006–1013