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## Regulation of VLDL synthesis and secretion in the liver

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**Summary** — The production of very low density lipoproteins (VLDL) by the liver results from very complex processes that involve coordinated mechanisms of both protein and lipid synthesis and packaging. Alterations in these metabolic functions can cause negative effects on the health of human subjects or animals. The objectives of this paper were to review the latest developments in the biological mechanisms of these processes and the role of nutritional and hormonal factors. The present study addresses the following issues: i) the main steps in the hepatic metabolism of lipids (long-chain fatty acids, triacylglycerols, phospholipids) and proteins (apolipoprotein B, microsomal transfer protein) primarily involved in the synthesis and secretion of VLDL particles; ii) the metabolic deviations of hepatic VLDL (hypo- and overproduction) in man, rodents and farm animals (poultry, dairy cows).

**liver / triacylglycerol / VLDL secretion / regulating factors / steatosis**

**Résumé** — **Régulation de la synthèse et de la sécrétion des VLDL par le foie.** *La formation par le foie de particules de lipoprotéines de très basse densité de type VLDL implique un processus très complexe qui nécessite la synthèse et l'assemblage coordonnés des constituants protéiques et lipidiques. Le dysfonctionnement de cette fonction hépatique peut provoquer des effets néfastes sur la santé des sujets. Le but de cette revue est de présenter les données les plus récentes et les concepts actuels concernant les mécanismes mis en jeu dans ces processus, ainsi que le rôle des facteurs de régulation de type nutritionnel et hormonal. Les points majeurs abordés dans cette synthèse concer-*

**Abbreviations:** apoB: apolipoprotein B; CE: cholesteryl esters; DAG: diacylglycerols; DGAT: diacylglycerol acyltransferase; ER: endoplasmic reticulum; FABP: fatty acid binding protein; FA: fatty acids; GPAT: glycerol-3 phosphate acyltransferase; IDL: intermediate density lipoproteins; LDL: low density lipoproteins; LCFA: long-chain fatty acids; LPA: lysophosphatidic acid; LPAT: lysophosphatidic acid acyltransferase; MTP: microsomal transfer protein; NEFA: nonesterified fatty acids; PA: phosphatidic acid; PAP: phosphatidic acid phosphatase; PC: phosphatidyl choline; PDI: protein disulphide isomerase; PL: phospholipids; PUFA: polyunsaturated fatty acids; RER: rough endoplasmic reticulum; TAG: triacylglycerols; VLDL: very low density lipoproteins.

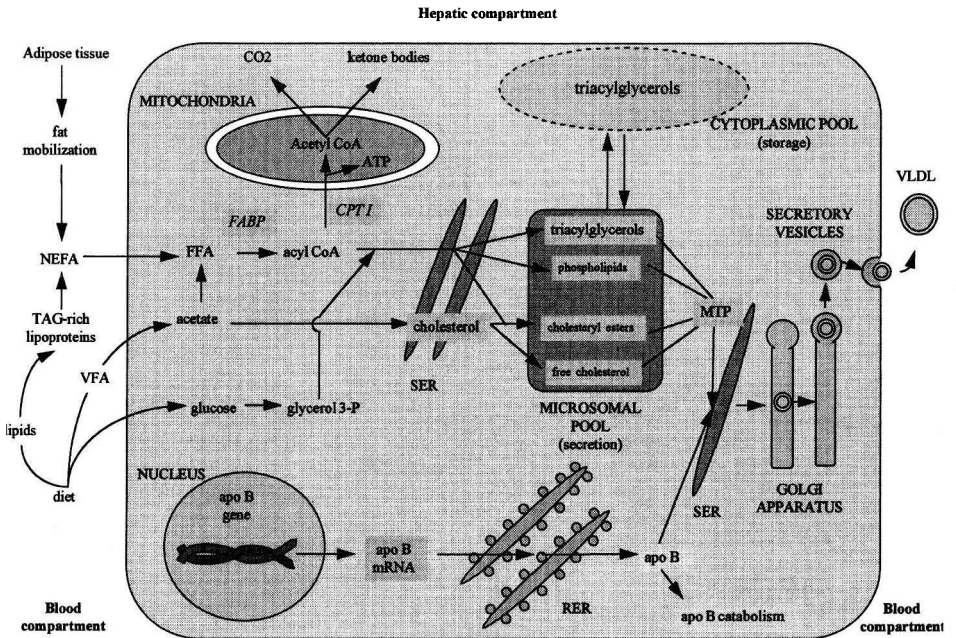
ment, d'une part, les particularités des principales étapes du métabolisme hépatique des lipides (acides gras, triglycérides, phospholipides) et des protéines (apolipoprotéine B, protéine microsomale de transfert des triglycérides) majoritairement impliquées dans la synthèse et la sécrétion des VLDL. La deuxième partie de cette revue décrit les mécanismes et les facteurs nutritionnels et hormonaux impliqués dans les déviations du métabolisme hépatique des VLDL (hypersécrétion, hyposécrétion) chez l'homme et les rongeurs de laboratoire et chez les animaux d'intérêt zootechnique (volaille, vache laitière).

**foie / triglycérides / sécrétion de VLDL / facteurs de régulation / stéatose**

**INTRODUCTION**

The production of very low density lipoproteins (VLDL) rich in apolipoprotein B (apoB) and triacylglycerols (TAG) by the liver involves complex processes such as synthesis and the coordinated assembly of protein components (apolipoproteins) and of hydrophilic (phospholipids, free cholesterol)

and hydrophobic (TAG, cholesteryl esters) lipid components (fig 1). This hepatic function enables mammals to transport efficiently a powerful source of energy either for storage in adipose tissues or for utilization, predominantly by muscle tissues. However, disorders in the liver lipotrope function may lead to diseases (overproduction of VLDL and coronary artery diseases, defect in VLDL



**Fig 1.** Schematic representation of the different processes involved in the synthesis and secretion of very low density lipoproteins (VLDL) in animal livers. apo B: apolipoprotein B; CPT I: carnitine palmitoyltransferase I; FABP: fatty acid binding protein; FFA: free fatty acids; MTP: microsomal transfer protein; NEFA: nonesterified fatty acids; RER: rough endoplasmic reticulum; SER: smooth endoplasmic reticulum; VFA: volatile fatty acids.

production and liver steatosis-cetoacidosis). Several reviews have described the different steps of synthesis, assembly and secretion of VLDL in the liver and the regulating factors involved (Gibbons, 1990; Vance and Vance, 1990; Glickman and Sabesin, 1994). The present paper details the recent developments and understandings of the mechanisms involved in the hepatic synthesis of VLDL and how these are affected by different nutritional and hormonal factors.

## **VLDL SYNTHESIS AND SECRETION IN THE LIVER**

### ***Fatty acid metabolism***

Long-chain fatty acids (LCFA) entering the liver are derived from plasma nonesterified fatty acids (NEFA) mobilized from adipose tissues during fasting conditions or from hydrolysis of dietary TAG during the post-prandial period. The hepatic uptake of NEFA is driven by mass action and therefore depends on their concentration and the rate of blood flow into the liver (Pethick et al, 1984). In the liver, LCFA can also be synthesized from acetyl-CoA produced by the catabolism of excess glucose. The metabolic fates of LCFA in hepatocytes are i) the esterification into TAG and, to a lesser extent, into phospholipids (PL) and cholesteryl esters (CE); ii) the complete oxidation to CO<sub>2</sub> generating ATP molecules or the incomplete oxidation to acetate and ketone bodies (fig 1) (Zammit, 1990). The metabolism of LCFA in the liver depends on the feeding conditions. In the fed period, carbohydrate ingestion favors esterification of LCFA by increasing availability of glycerol-3 phosphate produced from glucose and by inhibiting LCFA oxidation by maintaining high levels of malonyl-CoA, an inhibitor of carnitine transferase (McGarry and Foster, 1980). In the same way, increasing the supply of dietary LCFA to the liver stimulates esterifi-

cation of LCFA to TAG but affects their secretion as VLDL (Francone et al, 1992). In the fasting period, the hepatic level of malonyl-CoA decreases which eliminates LCFA oxidation inhibition (McGarry et al, 1978). Such a situation favors acetyl-CoA formation and its subsequent oxidation. The length and the degree of unsaturation of the exogenous fatty acid (FA) carbon chain entering the liver may also control the intrahepatic metabolism of these FA. Medium-chain FA raise hepatic TAG synthesis levels in rats (Geelen et al, 1995) whereas n-3 polyunsaturated FA (PUFA) are preferentially oxidized to ketone bodies (Rustan et al, 1992). FA metabolism in the liver is also under hormonal control. Insulin inhibits the oxidation of FA but stimulates the formation of LCFA from glucose and the synthesis of TAG from LCFA (Zammit, 1995).

### ***Triacylglycerol metabolism***

The hepatic synthesis of TAG generally proceeds along a sequence involving four reactions: three esterification reactions and one hydrolysis reaction. The first reaction is the acylation of glycerol-3 phosphate to acylglycerol-3 phosphate (ie, lysophosphatidic acid, LPA) which occurs on the endoplasmic reticulum (ER) or in the mitochondria by glycerol-3 phosphate acyltransferase (GPAT). In rat liver, the mitochondrial form of this enzyme (GAT, EC 2.3.1.15) has a specificity for saturated acyl-CoA whereas the microsomal form (GPAT, EC 2.3.1.15) apparently exhibits no FA specificity (Vancura and Haldar, 1994). It is not clear, however, whether the acyltransferase activity is due to a single enzyme with a dual specificity or to two enzymes with different specificities. Mitochondrial LPA is carried by the liver fatty acid binding protein (FABP) to the ER (Vancura and Haldar, 1992) where a second acylation, catalyzed by the lysophosphatidic acid acyltransferase (LPAT, EC

2.3.1.51) produces phosphatidic acid (PA). The third step of TAG synthesis consists of hydrolysis of the phosphate ester bond of PA, yielding diacylglycerols (DAG), by the phosphatidic acid phosphatase (PAP, EC 3.1.3.4) located in the cytosol and in the microsomes of hepatocytes. These three first reactions are the same as those of PL synthesis which mainly occurs in the ER. The fourth and last reaction is specific to TAG synthesis. It consists of the acylation of DAG to TAG by microsomal diacylglycerol acyltransferase (DGAT, EC 2.3.1.20). During the last 5 years, numerous studies have been carried out to specify the relative importance and the mechanisms by which dietary FA control TAG synthesis in hepatocytes. Recent investigations on rat hepatocytes have emphasized GPAT activity as the main limiting step controlling the overall flux of LCFA through the esterification pathway to PL or TAG (Stals et al, 1994), irrespective of the dietary LCFA composition (Rule, 1994). Dietary lipids, rich in n-3 PUFA (from fish oil), led to a decrease in GPAT activity that was positively correlated with the induction of hypotriglyceridemia (Willumsen et al, 1993). In fact, this decrease in TAG synthesis originated from the greater oxidation of n-3 PUFA, therefore reducing their availability for esterification (Willumsen et al, 1993). Data on the regulation of PAP and DGAT activities by dietary lipid sources are still conflicting. Several recent studies on rat liver revealed that diets containing fish or safflower oil reduced PAP activity more efficiently than diets containing palm or sunflower oil, without altering the DGAT activity (AL-Shurbajī et al, 1991; Halminski et al, 1991). More recently, Rustan et al (1992) demonstrated that the source of dietary PUFA (fish oil vs sunflower oil or linseed oil) has no effect on PAP activity whereas Willumsen et al (1993) indicated that eicosapentaenoic acid (EPA, C20:5n-3) from fish oil stimulated DGAT activity. Moreover, in rats fed fish oil, hepatic DGAT activity was significantly reduced when com-

pared with rats fed corn oil (Geelen et al, 1995). DGAT activity does seem to be modulated by the length of the FA carbon chain since diets containing medium-chain FA (mainly constituted of C8:0 and C10:0) could stimulate this activity more efficiently than diets containing corn oil (Geelen et al, 1995).

LCFA synthesized de novo in hepatocytes, lipoprotein remnants returning to the liver and plasma NEFA taken up by the liver are the potential sources of LCFA for the hepatic synthesis of TAG. It has been shown that hepatic de novo synthesis of LCFA provides only a small fraction of the newly secreted VLDL both in rats (Gibbons, 1990) and man (Hellerstein et al, 1991). On the other hand, many in vitro studies have clearly shown that extracellular LCFA (mainly NEFA) are capable of providing the bulk of nascent VLDL-TAG (Gibbons, 1990; Gibbons et al, 1992). Time-course studies carried out on rat hepatocytes in culture (Gibbons et al, 1992) and in vivo in the sheep liver (Pullen et al, 1988), however, suggest that the TAG synthesized from plasma NEFA are not immediately incorporated into the nascent VLDL particles, but are initially transferred into a temporary storage pool, probably located in the hepatocyte cytosol (fig 1). The precise pathways through which TAG are recruited from this pool to meet the requirement for VLDL assembly and secretion are still unknown. However, cytosolic TAG are not likely to be transferred as intact molecules into nascent VLDL particles but undergo lipolysis followed by re-esterification of the resulting LCFA in microsomes (Mooney and Lane, 1981; Wiggins and Gibbons, 1992). The chemical structure of the lipolytic factors involved in the TAG cycle, their catalytic properties and their precise location within the cell, are unknown. Data from different studies have only demonstrated that these lipolytic processes do not involve the activities of either lysosomal acid lipase, hormone-sensitive lipase or exogenous lipopro-

tein lipase absorbed onto hepatic sinusoids (Wiggins and Gibbons, 1992).

### ***Phospholipid metabolism***

Phosphatidyl choline (PC), the major phospholipid component of VLDL, is located exclusively on the hydrophilic surface of the particles. Inhibition of PC biosynthesis by choline deprivation in rat hepatocytes strongly reduces VLDL secretion (Yao and Vance, 1988). The requirements for active PC synthesis were shown to be highly specific since the addition of ethanolamine or monomethylethanolamine (which is converted into phosphatidyl monomethylethanolamine) into the culture medium of rat hepatocytes did not restore VLDL secretion (Yao and Vance, 1989). The biosynthetic origin of PC is not an important factor since conventional VLDL secretion by cultured hepatocytes can be restored by the addition of choline, methionine (which promotes PC biosynthesis via the methylation of phosphatidyl ethanolamine) or lyso-PC (which can be acylated to PC) (Robinson et al, 1989). The mechanism by which choline deprivation causes a reduction in the secretion of VLDL was investigated by Verkade et al (1993). These authors showed that the number of VLDL particles assembled in the ER was similar in choline-deficient and choline-supplemented rats, but that the Golgi fraction was lower in the choline-deficient animals only. These data suggest a possible protection mechanism for the neosynthesized apoB from intracellular degradation by choline during the migration of apoB from the ER to the Golgi apparatus (Verkade et al, 1993).

### ***ApoB synthesis***

Human apoB is a large, hydrophobic protein of 4 536 amino acids with a molecular

mass of approximately 520 kDa. This protein, termed apoB-100, is synthesized exclusively in the liver. ApoB-100 contains both hydrophobic lipid-binding regions, which participate in the assembly of nascent lipoproteins, and hydrophilic sequences which interact with the polar aqueous environment. ApoB-100 also contains an LDL-receptor binding domain that is involved in the uptake of plasma IDL and LDL (and possibly VLDL) by tissues (Young, 1990). Numerous studies support the concept that hepatic apoB-100 secretion is metabolically regulated. Various metabolic states such as food deprivation and carbohydrate overload alter the level of hepatic apoB secretion, although the amount of apoB-100 mRNA is generally constant in hepatocytes. *In vitro* studies showed that free FA (butyrate, oleate) or insulin (Dashti et al, 1989; Pullinger et al, 1989; Kaptein et al, 1991) also modulate apoB secretion without any modification of the apoB-100 mRNA level. These results indicate that, in most physiological or nutritional situations, apoB-100 mRNA, which has a relatively long half-life of 16 h (Pullinger et al, 1989), does not respond to acute stimuli. These observations have focused attention on the co- and posttranscriptional mechanisms that could modulate apoB-100 production (Adeli et al, 1995). In HepG2 cell and rat hepatocyte cultures, insulin and thyroid hormone modify the rate of apoB mRNA translation (Theriault et al, 1992a, b). Recent data clearly demonstrated a rapid degradation of newly synthesized apoB-100 (> 50%) in rat hepatocytes (Sparks and Sparks, 1990) and HepG2 cells (Sato et al, 1990). This process may regulate the number of molecules of apoB-100 that enter the secretory pathway (Borchardt and Davies, 1987). Despite numerous data that demonstrated the hepatic degradation of newly synthesized apoB, little is known about this degradative pathway and its regulation. Recently, Cartwright and Higgins (1995b) identified at least two intracellular sites of apoB degradation, the

ER and Golgi compartment, involving the activities of several proteases. In the *in vitro* degradation systems (permeabilized HepG2 cells), apoB catabolism was pH dependent and was stimulated by conditions of high temperature and by ATP. This proteolytic activity did not require calcium but was strongly influenced by the cysteine protease inhibitor (Adeli, 1994). The rate of apoB catabolism is stimulated by insulin (Sparks and Sparks, 1990) and decreased by oleate (Dixon et al, 1991; Furukawa et al, 1992). The number of newly synthesized apoB molecules available for secretion is modulated by the amount of TAG (Wu et al, 1994), CE (Cianflone et al, 1990; Dashti, 1992) and PL (Vance and Vance, 1990; Verkade et al, 1993) in the microsomes. The addition of oleate to the HepG2 or McArdle-RH777 cell medium highly stimulates apoB secretion (White et al, 1992; Sakata et al, 1993), which is correlated with an increase in the intracellular TAG level. The authors concluded that TAG would protect apoB through the formation of an apoB-TAG complex that is resistant to proteolysis (Furukawa et al, 1992; Sakata et al, 1993). Conversely, inhibition of TAG synthesis by triacsin D prevents this oleate effect and favors intrahepatic apoB degradation (Wu et al, 1994).

### ***Microsomal transfer protein***

The microsomal transfer protein (MTP) catalyzes the transport of TAG, CE and PL from the ER membranes to the site of VLDL assembly. Exclusively found in the lumen of microsomes isolated from the liver and intestine, this protein is a soluble heterodimer which consists of two subunits of 58 and 97 kDa, respectively (Gordon et al, 1995). The smaller subunit corresponds to a multifunctional enzyme, the protein disulphide isomerase (PDI), which plays several roles in protein folding within the lumen of

the ER. The precise role of PDI in the functioning of MTP is still unclear. The two subunits, however, form a tight, nonexchanging complex, and the dissociation of this complex leads to a loss in TAG transfer activity (Wetterau et al, 1991). The cDNA encoding the larger subunit of MTP has been cloned and sequenced from the livers of cows, humans (Sharp et al, 1993) and hamsters (Lin et al, 1994). The deduced amino acid sequence of the bovine and hamster MTP is 86% identical to that in humans, indicating high conservation of MTP between species. MTP has a specific preference for binding and transporting hydrophobic lipids (TAG, CE) over PL. Indeed, its lipid transport rates decrease as follow: TAG > DAG > free cholesterol > PC. Within a lipid class, MTP tends to transport molecules with lower polarity more easily (Jamil et al, 1995). The mechanisms of lipid transport by MTP between membranes have been studied in kinetic and lipid-binding experiments. It has been suggested that MTP shuttles lipids to the newly synthesized apoB in the lumen of the ER (Atzel and Wetterau, 1994). The expression of the gene encoding the large subunit of MTP is regulated by nutritional factors that alter the levels of *in vivo* VLDL synthesis by the liver (Lin et al, 1994). Thus, high-fat and high-sucrose diets given to hamsters for 1 month increased the hepatic large subunit mRNA level by 55% whereas a prolonged fast for 48 h had no effect (Lin et al, 1994). In the hamster, the hepatic level of MTP mRNA increased with high-fat rations and with high levels of saturated FA (C14, C16) as compared with unsaturated FA (C18:1n-9, C18:2n-6) in the diet (Bennett et al, 1995). The mRNA level for the MTP larger subunit in HepG2 cells decreased by up to 80% in response to increasing insulin concentrations (Lin et al, 1995). However, there was no acute effect of insulin on MTP activity in HepG2 cells owing to the long half-life of the MTP protein in the hepatocytes (4.5 days) (Lin et al, 1995).

### **VLDL assembly**

Although there is still considerable controversy over the details of the VLDL assembly sequential process, it is commonly accepted that hepatic VLDL assembly is broken down into two steps (Gordon et al, 1995). The first step consists of the translocation of apoB into the lumen of the RER (where apoB is folded) and subsequently into the initial lipid supply to form nascent, small and dense lipoprotein particles. The second step consists of the maturation of the particles through the incorporation of neutral lipids into their hydrophobic core.

ApoB is an extremely hydrophobic protein, but analysis of the amino acid sequence indicates a lack of membrane-spanning sequences (Knott et al, 1986) at the difference of membrane proteins. However, it has been shown that apoB is cotranslationally bound to the ER membrane exposing a substantial portion of the molecule to the cytoplasmic side (Chuck and Lingappa, 1992; Boren et al, 1993), thus suggesting that apoB might require a novel translocation process. Thus, Chuck and Lingappa (1992, 1993) identified two distinct sequences in apoB (residues 86–99 and 305–318) which appeared to transiently 'pause' the translocation of apoB and stalled apoB in the translocation pore of the ER membrane. Then, the translocation should subsequently restart. The delay that occurs during the translocation process allows lipids to be added to the lipid-binding domains after their shuttle from the ER membranes to the growing apoB peptides in the lumen of the ER via MTP activity. The apoB molecules can, then, fold into a thermodynamically stable conformation before additional sequences are translocated into the lumen of the ER. The relationship between the translation/translocation process of apoB and the production of lipoproteins has been investigated in a series of pulse-chase experiments (Boren et al, 1992). Clearly,

nascent polypeptides of apoB, of which molecular weight reached 80–200 kDa, were associated with a unique type of lipoprotein. The size of the nascent polypeptides determined the size and the lipid load of the lipoprotein particles. It has recently been shown that nascent lipoproteins in the RER of rat livers already contain sufficient lipids to have diameters in the range of those of native VLDL. These results demonstrate clearly that the formation of apoB-lipoprotein is coupled not only to the translocation of the protein, but also to the translation of this molecule (Dixon and Ginsberg, 1993; Russiñol et al, 1993; Cartwright and Higgins, 1995a).

### **METABOLIC DEVIATIONS**

Disorders in lipoprotein metabolism can result from abnormal synthesis, processing or catabolism of plasma lipoprotein particles. Abnormal hepatic synthesis of VLDL can lead to hyper- or hyposecretion of these TG-rich lipoproteins in humans and in other mammals, especially farm animals.

#### ***VLDL hypersecretion***

Hypersecretion of VLDL is associated with an increased risk of cardiovascular diseases in primates (Alaupovic et al, 1990). These metabolic deviations are generally observed in humans with hormonal (noninsulin-dependent diabetes) or nutritional (obesity) disorders. However, the causes of VLDL hypersecretion have not actually been identified.

#### **Noninsulin-dependent diabetes**

Overproduction of hepatic VLDL particles is frequently observed in humans with a noninsulin-dependent form of diabetes characterized by tissue resistance to insulin.



This metabolic deviation is not correlated with a hypersynthesis of apoB since the VLDL particles generally exhibit abnormal size and altered chemical composition. Several metabolic abnormalities associated with a noninsulin-dependent form of diabetes, such as hyperglycemia, high plasma level of NEFA and hypoglucagonemia, are each associated with increased hepatic VLDL secretion (Howard, 1987).

### **Obesity**

Obesity is also associated with the hypersecretion of VLDL by the liver. In this metabolic disease, apoB and TAG secretions both increase to the same extent, leading to higher production of VLDL particles having unchanged size and chemical composition (Gibbons, 1990). Higher hepatic availability and utilization of NEFA for the synthesis of VLDL lipids (Egusa et al, 1985) and insulin resistance frequently observed in obesity can partly explain the hepatic hypersecretion of VLDL observed in obese patients.

### **VLDL hyposecretion**

Hyposecretion of VLDL in the liver results from the abnormal synthesis of lipoprotein particles. Epidemiologic studies have shown that patients with such disease have, on average, a lower risk for atherosclerotic cardiovascular disease but a higher risk for a variety of cancer, pulmonary or gastrointestinal diseases (Schonfeld, 1995). Fatty liver arises when an imbalance exists between the hepatic TAG formation and the synthesis and/or secretion of VLDL. This imbalance results from alterations in metabolic mechanisms (table I) generally caused by genetic, hormonal or nutritional factors.

### **Genetic diseases**

VLDL hyposecretion may result from different genetic disorders that lead to hypobetalipoproteinemia (Schonfeld, 1995). Mutations in the apoB gene that impede apoB synthesis or generate several nonfunctional truncated apoB molecules, and mutations in the gene of the large subunit of MTP (Sharp et al, 1993) that lead to an abnormal protein, have been observed in several cases of abetalipoproteinemia in humans (Zannis, 1989; Schonfeld, 1995).

### **Insulin-dependent diabetes**

Insulin-dependent diabetes reduces VLDL output by stimulating the degradation of newly synthesized apoB by insulin (Van Steenberghe and Lansckmans, 1995). Among other nutritional factors that also reduce VLDL secretion, fish oils (rich in n-3 PUFA) increased peroxisomal FA oxidation in hepatocytes, altering the availability of LCFA for TAG synthesis and secretion (Rustan et al, 1992).

### **Alcohol and liver steatosis (fig 2a)**

The excessive consumption of ethanol is frequently accompanied by malnutrition. Consequently, such dietary habits limit FA absorption but favor a higher mobilization of FA from adipose tissues which are then taken up by the liver. Excess ethanol impairs the mitochondrial oxidation of LCFA, leading to a higher amount of LCFA available for esterification into TAG. When the uptake of exogenous LCFA by the liver exceeds the ability of the liver to assemble and/or secrete the VLDL particles, a fatty liver can then develop (Baraona and Lieber, 1979). Excessive ethanol consumption also causes numerous injuries to the hepatocytes which may impair their protein synthesis, thereby interfering with the availability of apoB. At the subcellular level, ethanol can affect one

**Table I.** Possible mechanisms involved in the development of liver steatosis.

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- i. Increased supply of LCFA to the liver resulting from:
    - higher LCFA mobilization from adipose tissue
    - higher LCFA synthesis in the liver
    - lower LCFA oxidation in the liver
  
  - ii. Impaired TAG incorporation into VLDL:
    - through inhibition of apoB or MTP synthesis
    - through production of an inadequate supply of apoB or MTP molecules
    - through higher intracellular degradation of apoB
    - through competition with CE
  
  - iii. Defects in one or several steps in VLDL transport and secretion
    - through alterations of VLDL transport from ER to Golgi complex
    - through impairment of final apoB glycosylation in Golgi apparatus
    - through lower rate of secretory vesicles formation
    - through impaired migration of secretory vesicles from Golgi apparatus to cell membranes
- 

Abbreviations given on title page.

or several steps in intracellular transport, packaging or secretion of VLDL particles. These include defects in the movement of VLDL out of the Golgi apparatus associated with an interference between the ethanol and microtubule formation (Baraona and Lieber, 1979). Alcoholic liver disease may be prevented by the supply of adequate nutrients (Derr and Gutmann, 1994). A study in the rat reported that, during prolonged alcohol consumption by rats, a high level of unsaturated fats and low energy in the diet favored fatty liver formation whereas higher energy intake reduced lipid infiltration (Sankaran et al, 1994).

#### **Parenteral nutrition and steatosis (fig 2b)**

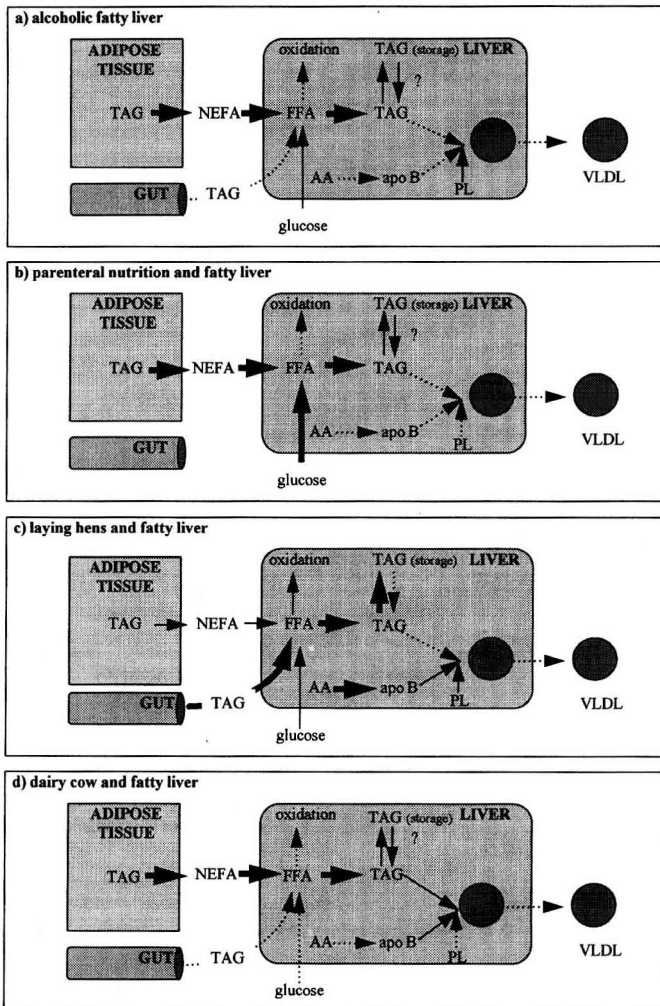
Fatty liver is a common abnormality in patients given total parenteral nutrition (Fisher, 1989). Development of the syndrome appears to depend on the amount

of glucose infused. Plasma infusion of glucose leads to hyperinsulinemia, which inhibits VLDL secretion. Among the possible explanations is that the accumulation of TAG in the hepatocytes may result from higher peripheral lipolysis, lower LCFA oxidation, higher de novo LCFA synthesis and esterification to TAG, and also higher apoB degradation which impairs the VLDL production. Total parenteral nutrition may provide excess calories with glucose, associated with an inadequate protein or amino acid supply. The addition of a lipid emulsion to parenteral nutrition lowers the portal insulinemia and the portal molar ratio of insulin to glucagon, leading to a reduction in lipid infiltration into the rat liver (Nussbaum et al, 1992). Moreover, it was shown that hepatic steatosis in patients receiving long-term parenteral nutrition was induced by a plasma-free choline deficiency and was reversed using lecithin supplementation (Buchman et al, 1992).

### Poultry and steatosis (fig 2c)

Fatty liver disease in laying hens is associated with an hemorrhagic syndrome that is induced by a definitive failure of liver reticulin integrity (Hansen and Walzem, 1993). During egg production, high levels of plasma estrogen induce an increased food intake and a state of positive energy balance in the hen. Such metabolic situations favor synthesis of LCFA, TAG and apoproteins

in the liver (Dashti et al, 1983). A majority of the neosynthesized TAG, however, is stored in a cytoplasmic pool and probably needs to be hydrolyzed and reesterified into TAG before being incorporated into VLDL particles (Mooney and Lane, 1981). This hydrolysis of TAG is stimulated by food deprivation. The typical ingestive behavior of hens, however, results in a nearly constant fed state. This constant fed state can limit the transfer of TAG from the cytoplasmic stor-



**Fig 2.** Schematic representation of the metabolic pathways involved in fatty liver development in humans and animals: **a)** alcoholic fatty liver; **b)** parenteral nutrition and fatty liver; **c)** fatty liver in laying hens; **d)** fatty liver in dairy cows. The large arrow (→) indicates the metabolic pathway that is activated and the dotted arrows (--->) represent the metabolic pathway that is deactivated. AA: amino acids; apo B: apolipoprotein B; FFA: free fatty acids; NEFA: nonesterified fatty acids; PL: phospholipids; TAG: triacylglycerols; VLDL: very low density lipoproteins.

age pool to the secretory microsomal pool and their subsequent incorporation in VLDL particles. The chronic and positive energy balance observed in laying hens maintains a relatively high insulinemia (Simon, 1989) which favors the inhibition of VLDL secretion (Simon, 1989). Geese that have been force-fed for 2 weeks with a carbohydrate-rich diet develop dramatic liver steatosis leading to a large increase in liver weight (ten-fold). The induction of noteworthy fatty liver is partly under genetic control but the mechanisms of steatosis development are still poorly understood (Hermier et al, 1991). Force-feeding favors hepatic lipogenesis in the goose and produces higher plasma levels of VLDL. These particles, however, are especially enriched in CE, indicating either a defect in the incorporation of TAG into nascent hepatic VLDL particles or a competition between hydrophobic lipids during VLDL packaging (Hermier et al, 1991). Alterations in hepatic secretion of TAG would therefore stimulate liver steatosis.

### **High producing dairy cows and steatosis (fig 2d)**

Fatty liver is generally seen as a peripartum metabolic disorder that affects one-third of periparturient, high-producing dairy cows (Grummer, 1993). Maximal TAG infiltration occurs between 2 and 4 weeks postcalving (80–100 mg/g fresh liver vs 2 mg/g fresh liver in dry cow) and is the result of excessive FA mobilization when the energy intake is insufficient to support both maintenance and milk production. It disappears after 12 weeks when positive energy balance is restored (Grummer, 1993; Durand et al, 1994).

Another major factor contributing to the development of fatty liver is the chronic slow output of hepatic TAG, which form part of the VLDL particles (Durand et al, 1992; Bauchart, 1993; Grummer, 1993). In early lactation, a decrease in plasma apoB con-

centration is correlated with an increase in liver TAG content (Marcos et al, 1990). Similar induction of fatty liver was obtained when ethionine (an inhibitor of protein synthesis) was given to cows, suggesting that apolipoprotein synthesis is a limiting step for VLDL secretion (Uchida et al, 1992). Simultaneous determinations of hepatic levels of apoB, apoB mRNA and TAG clearly showed a negative correlation between apoB gene expression and fatty liver development in the first week of lactation (Gruffat et al, 1994). In other respects, the impairment of the signal transduction pathway mediated by protein kinase C appears also to be involved in the pathogenesis of fatty liver in cows (Kato, 1994). Different results suggest a hepatic deficiency in amino acids and in phospholipid precursors in high producing dairy cows in early lactation. Nutritional treatments such as intravenous infusions of L-methionine and L-lysine (Durand et al, 1992) or choline (Juslin et al, 1965), as precursors for PC synthesis, to cows in early lactation favored hepatic VLDL secretion and reduced lipid infiltration. Extensive hydrogenation of dietary PUFA by the rumen bacteria is probably a positive factor that contributes to limiting the extension of hepatic steatosis in dairy cows. Thus, substitution of soybean oil (rich in n-6 PUFA) for tallow rich in saturated and monounsaturated FA in milk replacers for preruminant calves (functional monogastric animals) favored, as in force-feeding goose (Hermier et al, 1991), the hepatic secretion of VLDL enriched in CE to the detriment of TAG which accumulated in the liver (Leplaix-Charlat et al, 1996a, b).

### **CONCLUSION**

This paper reviews data and new concepts on the complex processes involved in VLDL synthesis and secretion in the liver. While the major components of VLDL and their

pathways synthesis are broadly understood, many aspects of the molecular and cellular mechanisms involved in the assembly and secretion of VLDL particles and their regulation factors need further clarifications. For example, the mechanisms that regulate i) TAG transfer from the cytosolic storage pool to the microsomes; ii) hepatic catabolism of newly synthesized apoB catabolism (involving activity of proteolytic factors); iii) packaging of VLDL components require further investigations to propose adapted treatments against disorders resulting from hepatic over- or hyposecretion of VLDL. Thus, nutritional treatments capable of reducing the development of steatosis, such as balancing the dietary amino acids and dietary long-chain fatty acids and phospholipid precursors, should be examined more thoroughly. These are only a few of the many important questions that need further investigation.

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