

Gene Therapy for Bovine Fatty Liver : Possibilities and Problems - A Review

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ABSTRACT : Dairy cows are prone to fatty liver during the time of periparturient. Despite of the extensive studies, etiology and solutions for fatty liver are still not well known. The liver synthesizes triglycerides (TG) using precursors from bloodstream and secretes TG in form of very low density lipoprotein (VLDL) into bloodstream for the utilization by peripheral tissues. When the amount of TG synthesis exceeds the amount of secretion in VLDL-TG, TG accumulation within the liver occurs. Hepatic VLDL assembly and secretion involve multi-biochemical events. The availabilities of apolipoprotein B (apoB), E (apoE), microsomal triglyceride transfer protein (MTP) and soluble low density lipoprotein (LDL) receptor are now believed to be some of the main regulators for hepatic VLDL assembly and secretion. Studies in transgenic animals show that overexpression of these proteins stimulates VLDL production and secretion, which provides a possibility for alleviating bovine fatty liver by gene therapy. However, many problems remain to be solved to attain this goal. This review focuses on the molecular mechanisms of hepatic VLDL assembly and secretion, and the possibilities and problems of applying the knowledges to solve bovine fatty liver by gene therapy. (*Asian-Aust. J. Anim. Sci. 2001, Vol 14, No. 9 : 1331-1341*)

Key Words : VLDL, Fatty Liver, Gene Therapy, Bovine

INTRODUCTION

Fatty liver is one of the serious metabolic disorders that dairy cows suffer during the periparturient period. This disorder is associated with dysfunctions of the liver and the decrease of milk production and reproductive efficiency, and therefore the loss of economic benefit. Extensive epidemiological studies, especially in the aspect of nutrition, unreveal the reason and the underlying mechanism(s) for fatty liver development. Effective solutions have not yet been available for alleviating this disorder. Continuous studies in more basic levels are of great values for both academic and practical views. The objective of this review is to present the main molecular events of hepatic triglyceride secretion and to explore the possibility and problems of gene therapy for bovine fatty liver.

CURRENT UNDERSTANDING OF BOVINE FATTY LIVER

Fatty liver syndrome, or liver steatosis, characteristic of excessive lipid accumulation in the liver, is a metabolic disorder which occurs usually during periparturient period from 2-3 weeks before calving to 4-5 weeks after calving of dairy cows, especially high producing cows (figure 1). Reid (1980) reported the incidence of moderate (3-5% TG in fresh liver) and severe (more than 5% TG in fresh liver) fatty liver reached up to 30% in the early lactation immediately prepartum up to 6-8 weeks postpartum in high producing dairy cows. More recently, Grummer (1993)

summarized three studies from 61 multiparous Holstein cows and indicated that the average liver triglyceride (TG) content on dry matter (DM) basis at 17 day prior to calving, day 1 and 2 postpartum, and day 21 to 35 postpartum ranged from 2.1 to 7.1, 14.3 to 23.2 and 16.0 to 26.9% respectively. Liver TG peaks at or near calving. 50% of the 61 cows had more than 15% liver TG by d 1 postpartum.

It is believed that the development of bovine fatty liver is associated with the excessive mobilization of adipose tissue resulting from the decline of voluntary feed intake during periparturient (figure 2). However, nutritional strategies taken to counteract the negative energy balance, such as feeding rapidly fermentable carbohydrates prior to parturient (Harmon et al., 1985; Grummer, 1995), adding fats into diets (Grum et al., 1996; Skaar, 1989), adding antilipolytic or lipotropic compounds (Skaar et al., 1989; Gerloff et al., 1986), may not always be effective for alleviating fatty liver.

Fatty liver is one of the consequences of the changes of many metabolic events during periparturient period. These changes are referred to as homeorhetic regulation (Bell and Bauman, 1996). The physiological significance of homeorhesis is to meet the substantial increase of the mammary nutrient requirements for milk production by repartitioning external and endogenous nutrients away from nonmammary tissues towards mammary tissue. Since the declined feed intake is unable to meet mammary needs, the mobilization of adipose tissue is inevitable. As a result, plasma nonesterified fatty acids (NEFA) are enhanced for several folds (figure 2). About 20% of mobilized lipid is utilized by mammary gland for milk fat synthesis. Majority of the remaining NEFA is taken up by the liver (Bell and Bauman, 1996).

In the liver, fatty acids are first activated to acyl-CoA by

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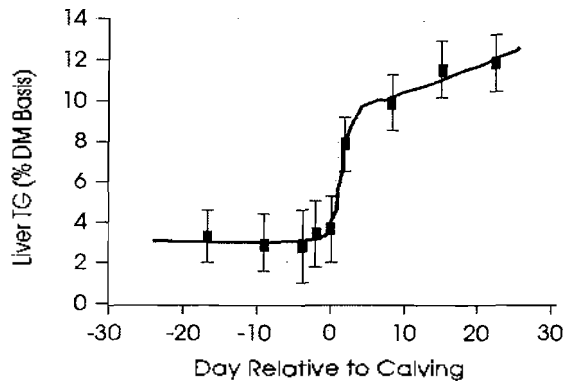


Figure 1. Changes in liver triglyceride concentration relative to calving (From Vazquez-Anon et al., 1994)

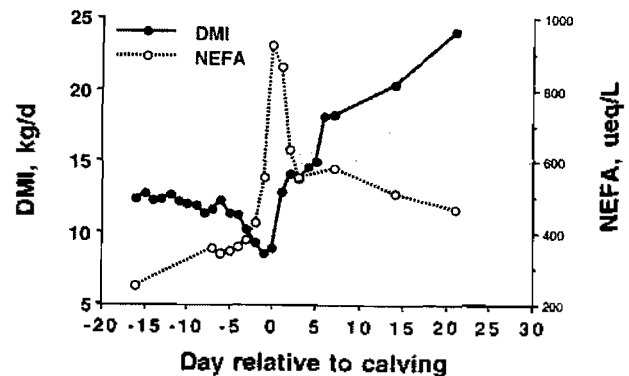


Figure 2. Dry matter intake (DMI) and plasma NEFA concentration of dairy cows during periparturient period (From Bertics et al., 1992)

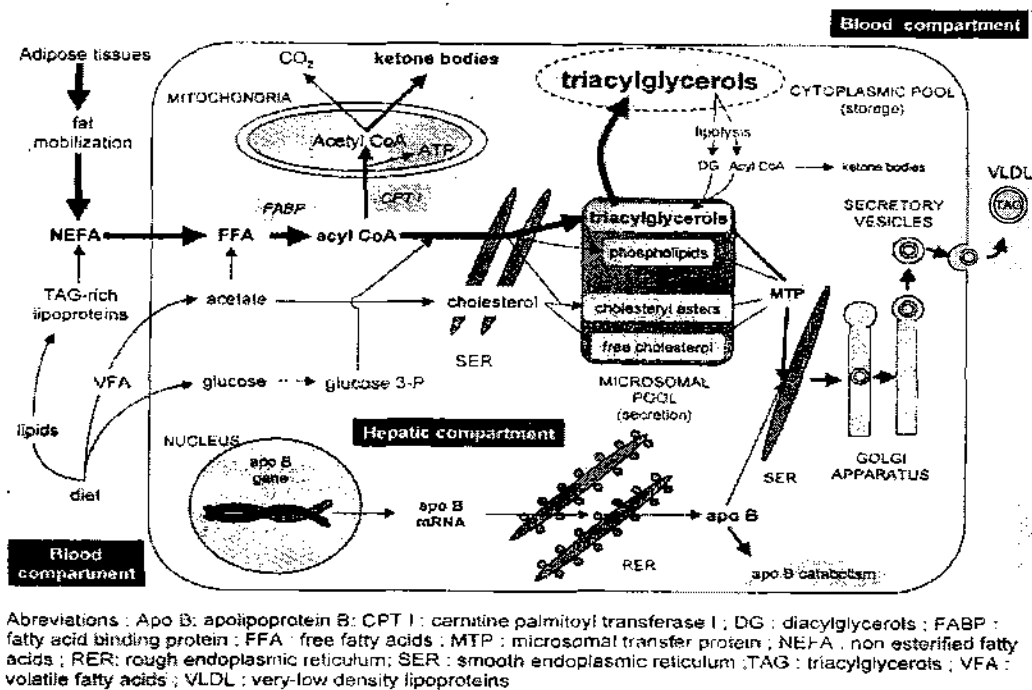


Figure 3. Metabolic pathways in dairy cows during peripartum fatty liver (From Gruffat et al., 1996)

the acyl-CoA synthase (figure 3). Acyl-CoA can be either oxidized completely or partially in mitochondria to produce energy, CO_2 or ketone bodies, or esterified to form TG, phospholipids or cholesteryl esters in the cytoplasm. After esterification, TG can be stored in the cytoplasm of the hepatocytes in the form of fat droplets, which can be hydrolyzed by lysosomal lipase for oxidation or re-esterification (Wiggins, 1992). The alternative fate of TG in the liver is to be exported in the particle of VLDL (Brody, 1999).

Although the metabolic pathways of lipids in the liver are well defined (figure 3), the rate-limiting steps of TG

export in transition cows are not yet precisely identified. In ruminants, the rate of esterification is similar to other species, but the rate of secretion of TG in the form of VLDL is very low (Pullen et al., 1990). Klepp et al. (1988) found similar rates of incorporation of oleate into TG by goat and rat hepatocytes, but the rate of TG secretion as VLDL was 20 times lower in goats than in rats. The difference must be greater during peripartum than other stages because of the higher hepatic diacylglycerol acyltransferase (DGAT) activity (van den Top, 1995) and lower hepatic ApoB (Gruffat, 1997) during peripartum. Why TG rich VLDL synthesis and secretion is low in

ruminants is not clear. Some of the explanations proposed currently include the low lipolysis of stored TG, which approximately accounts for 70% of sources for secreted TG (Gruffat et al., 1996; Wiggins, 1992), the low ratio of phospholipid to TG (Fronk, 1980), and the insufficiency of apolipoproteins (Gruffat, 1997). Obviously, understanding the mechanism of VLDL synthesis and secretion is a prerequisite for understanding fatty liver development and developing therapeutic strategies.

THE MOLECULAR BASIS OF VLDL SECRETION

VLDL is composed of TG, cholesterol, cholesteryl ester, phospholipid and proteins with total lipids accounting for 90%, of which 55-65% is TG, and proteins for 10% in humans (Brody, 1999). Different species of animals have a little bit different in percentage of each component in VLDL (Grummer and Carroll, 1988; Chapman, 1980). All hydrophobic components (TG and cholesteryl ester) and regions of amphipathic components (phospholipid, cholesterol) are located within the core, and hydrophilic components (proteins) or regions make up the outer surface of VLDL particles. The make-up proteins include apolipoprotein B (apoB), E (apoE) and C (apoC). It is believed that apoC is largely required after entry of VLDL into plasma, it is not involved in VLDL synthesis and secretion. Indeed, studies in humans and mice reveal that apoB is the sole apolipoprotein required for VLDL assembly (Shelness et al., 1999). There are two kinds of apoB, apoB100 and apoB48. In humans, the liver secretes only apoB100 and the intestines, only apoB48. But in rats, mice, dogs and horses, the liver can secrete both apoB100 and apoB48 (Brody, 1999).

Since the lipids and apoB for VLDL are synthesized at different compartments of the cell, the former in the smooth endoplasmic reticulum (ER) and the latter at the rough ER, apoB translocation is the very important event for VLDL assembly. It is accepted that VLDL is assembled by two steps (figure 4). During the first step, newly translated apoB polypeptide is co-translationally associated with a small amount of lipids and translocated from the cytoplasmic side of the rough ER to the luminal side across ER membrane. Microsomal triglyceride transfer protein (MTP) mediates this process (Shelness et al., 1999; Gordon, 1997). The product of this step is a dense, partially lipidated precursor of VLDL particles, i.g. nascent VLDL particles. The second step is to add bulk quantities of TG, cholesteryl esters and phospholipids to the VLDL precursor to form mature VLDL particles. MTP is not involved in the second step to a major extent (Rustaeus et al., 1999). This step is completed in different subcellular compartments for different species of animals. In rats, it is confined within ER (Rusinol et al., 1993), whereas in rabbits and chicken it is in

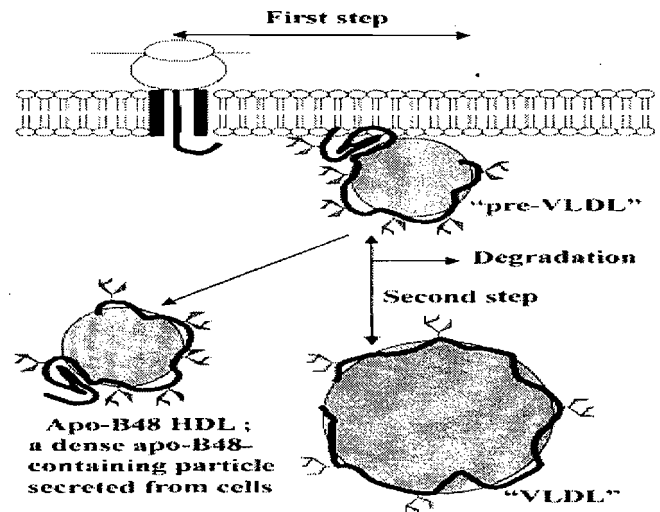


Figure 4. Two steps for VLDL assembly.

The first step is the formation of a VLDL precursor. This pre-VLDL can either go through the second step to form a VLDL particle or be degraded. In contrast to apoB100 VLDL precursor particles, apoB48 VLDL precursor particles can also be secreted. (From Rustaeus, S. et al., 1999)

the Golgi apparatus instead of ER (Bamberger and Lane, 1990; Gartwright, 1995).

Hepatocytes can synthesize excessive amounts of apo B, much more than the amount of its secretion. However, up to 65% of newly synthesized apo B undergoes intracellular degradation within ER (Yao et al., 1997), indicating that the regulation of apoB availability occurs mainly post-translationally. The extent and cellular site of degradation may vary with different hepatic systems. For example, apo B degradation occurs in the early stage of lipoprotein assembly in human hepatoma cells, while it does in both the early and late stages of secretion in primary hepatocytes (Ginsberg, 1995). This difference may reflect the different importance in lipid metabolism, especially in the ability to form and export VLDL of hepatic systems at different functional situations. During the whole process of VLDL assembly, many factors can affect the rate of apoB for degradation or secretion.

The translocation of apoB and the available activity of MTP are the crucial factors for the first step of VLDL assembly, determining the amount of apoB or VLDL secretion (Davis, 1997; Yao, 1994; Yao et al., 1997). ApoB is so hydrophilic that it must associate with lipids before its translocation. The newly synthesized apoB which fails to associate with lipids and translocate, undergoes rapid degradation by cytosolic proteasome or luminal proteases. (Shelness et al., 1999; Yao et al., 1997; Sparks and Sparks, 1993). Adding to this conclusion is that the newly synthesized apoB that is not used to make up nascent

particles after translocation is also subject to being degraded as Ingram and Shelness (1996) found apoBs destined for lipoprotein assembly and intracellular degradation are both translocated efficiently across the ER membrane.

MTP is a heterodimer that consists of a multifunctional protein-disulfide-isomerase and a unique 97-k DA subunit, the latter is responsible for the transport of neutral lipid between intracellular membrane (Gordon, 1997; Wetterau et al., 1990). It is located in the lumen of ER and appears to be essential for hepatic secretion of VLDL (Shelness et al., 1999; Jamil et al., 1998). It is assumed that the mechanism is to transfer lipids from the lumen of ER to apoB during its initial translation and to promote the association of apoB with lipids and the translocation of apoB (Tietge et al., 1999). Therefore, MTP seems to be the rate-limiting factor for hepatic VLDL secretion (Jamil, 1998). However, the short apoBs with molecular length less than 60% (Yao et al., 1997) or 25% (Shelness et al., 1999) of apoB-100 may be little dependent or independent of MTP activity for their translocation or secretion in contrast to apoB-100 and other large truncated apoB. The physiological significance of this difference is not yet known.

The VLDL precursor may also be degraded (figure 4), depending on the availability of lipids for further lipidation in the second step of VLDL assembly. The availability of the required lipids and the key enzymes required for the lipid syntheses can obviously regulate hepatic VLDL assembly and secretion (Davis, 1997; Kohen et al., 1995; Wu et al., 1994; Dixon and Ginsberg, 1993; Furukawa and Hirano, 1993; Verkade et al., 1993; Cianflone et al., 1990). The active synthesis and the mobilization of specific lipids to the site of assembly may be the most crucial physiological regulatory factors affecting the proportion of nascent apoB polypeptides that are secreted or degraded. Increased availability of neutral lipids, for example, facilitates the translocation of nascent apoB across the ER, decreases the posttranslational degradation of the protein, and enhances secretion of mature apoB-containing VLDL particles by the liver (Pease and Leiper, 1996; Yao and Mcleod, 1994). The saturation degree of dietary fat may also have some effects on VLDL secretion (Abdel-Fattah et al., 1995).

The role of cholesterol in the regulation of apo B secretion by the liver is reviewed by Thompson (1996). Although there are some conflicting evidences, an adequate availability of cholesterol, especially cholesteryl ester is a prerequisite for apoB secretion. It is believed that cholesteryl ester, together with TG, plays an essential role in the formation and stabilization of the lipid core of nascent VLDL particles through the mediation process by MTP.

However, although the availabilities of TG, cholesterol

or cholesteryl ester and phospholipid are all related with hepatic apoB-containing VLDL secretion, their availabilities are sufficient to support maximal apoB secretion at normal situations. Only when their availabilities fall below certain threshold level can the secretion of apoB or apoB-containing VLDL be affected (Dixon and Ginsberg, 1993). Therefore, it seems that VLDL secretion depends more on the availability of apoB itself and other apolipoproteins, the most probable one of which is apoE, for the assembly of VLDL.

ApoE is a 35000 dalton glycoprotein found in several lipoproteins (Mahley, 1988). It is synthesized mainly in the liver, but also present in many other tissues (Fielding, 2000; Lenich, 1988; Williams, 1985; Driscoll and Getz, 1984; Blue et al., 1983). ApoE plays important roles in the metabolism of TG and cholesterol, specifically in the clearance of plasma remnant lipoproteins by mediating the interaction of lipoproteins with hepatic and extrahepatic LDL receptors and hepatic apoE receptors, that is, LDL receptor-related proteins (Mahley, 1999, 1988; Ishibashi, 1994; Hui et al., 1984; Kita et al., 1982; Sherill et al., 1980). Thus apoE mutation will impair the clearance of plasma TG and cholesterol.

In addition to the well-established role in mediating uptake of lipoproteins, apoE may also play a role in facilitating hepatic VLDL assembly and secretion. Triglycerides are accumulated in the liver of apoE-deficient mice (Kuipers et al., 1996). Hepatic VLDL triglyceride production is impaired in apoE-deficient mice (Kuipers et al., 1997). However, when apoE-deficient mice overexpress chronically (Huang et al., 1998) and acutely (Tsukamoto et al., 2000) human apoE gene in the liver, hepatic VLDL triglyceride production significantly increases. On the other hand, the introduction of human apoE3-Leiden, a mutant apoE isoform, in mice resulted in the impair of hepatic VLDL-triglyceride secretion and the development of fatty liver (Mensenkamp et al., 2000). The regulatory role of apoE in hepatic VLDL triglyceride secretion is independent of its role in lipoprotein clearance (Mensenkamp et al., 1999).

The mechanism by which apoE participates the regulation of hepatic VLDL secretion is unknown. It may involve in the lipidation of nascent VLDL particles during the second step of VLDL assembly (Mensenkamp et al., 1999). Attie (1999) (personal communication) hypothesizes that apoE and apoB, while in the secretory pathway or on the cell surface in hepatocytes, compete for binding to the LDL receptor. ApoE is a better ligand and therefore displaces apoB, leading to less retention and degradation and more secretion of apoB (figure 5).

There are three isoforms of apoE in humans: apoE2, E3 and E4. Different isoform may have different biological effect (Mahley, 1988). ApoE3 is by far the most frequently

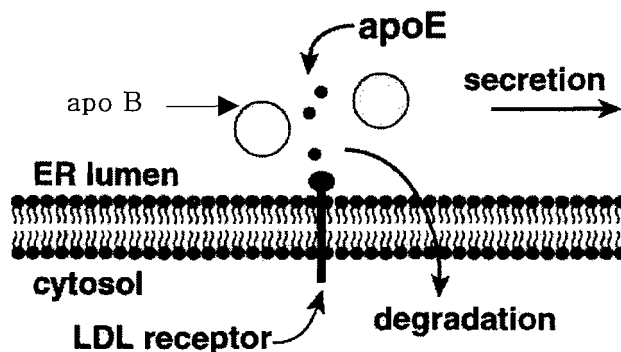


Figure 5. Model to explain the relationship between apoE expression and apoB secretion

occurring and binds normally to LDR. The biological effects of apoE3 depend on the extent of its expression (Huang et al., 1999). As apoE2 is defective in binding to LDR, apoE2 phenotype is subject to hyperlipoproteinemia although it has lower hepatic apoB secretion in form of VLDL than apoE3 phenotype (Demant et al., 1991). ApoE4 phenotype has a high secretion of VLDL-apoB due to the high supply of lipid substrate to the liver (Davignon, 1988). But, Tsukamoto et al. (2000) did not find the difference among the three isoforms in terms of hepatic secretion of VLDL in transgenic mice.

There are conflicting reports about the manipulation of dietary components on apo E synthesis. In cultured hepatocyte systems, the addition of fatty acids or glucose stimulates TG synthesis but does not change apo E synthesis or its mRNA levels (Davis and Boogaerts, 1982; Davis et al., 1985). However, Boogaerts et al. (1984) reported that feeding sucrose to rats increased both lipogenesis and apoE synthesis. Kim (1989) found only fasting and refeeding glucose after fasting were able to increase significantly hepatic apoE synthesis or mRNA levels of rats. Dietary fats, fructose had no effects. In Zanni et al. (1986)'s report, dietary saturated fat and cholesterol can modulate the apo E distribution within lipoproteins in rhesus monkeys.

LDL receptor (LDR) may be another regulator of hepatic VLDL secretion. It is well known that LDR and other members of LDR family play a critical role in the clearance of remnant lipoproteins from plasma (Hussain et al., 1999; Swamakart et al., 1998). However, patients with LDR deficiency (familial hypercholesterolemia) overproduce VLDL (James et al., 1989). LDR-deficient mice expressing SREBP-1a (a transcription factor for genes of lipogenic enzymes) have hyper-triglyceridemia due to the increase of hepatic VLDL production, whereas LDR-normal mice expressing SREBP-1a have severe fatty liver (Shimano et al., 1996; Horton, 1999). These observations imply a possible relationship between LDR and apoB

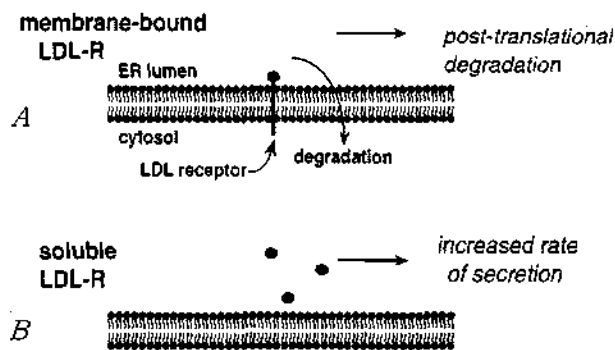


Figure 6. Relationship between LDL receptor(LDL-R) and the rate of apoB secretion

A: The native membrane-bound receptor interacts with apoB and targets it for degradation.

B: The soluble receptor binds to apoB and increases its rate of secretion.

secretion. Twisk et al. (2000) provided the direct evidence for this relationship for the first time. They found that LDR-deficient mouse hepatocytes secreted apoB100 at a 3.5-fold higher rate than wild-type hepatocytes did. There were no differences in apoB mRNA, apoB synthesis and MTP abundance between the two types of hepatocytes. Pulse-chase analysis showed much higher percentage of newly-synthesized apoB100 was degraded in wild-type hepatocytes compared to LDR-deficient hepatocytes. But overexpression of LDR in LDR-deficient hepatocytes increased presecretory degradation. This study shows LDR can regulate the proportion of apoB that escapes co- or post-translational presecretory degradation.

Dr. Attie' lab (personal communication) provides a further finding that only the LDR bound to the ER membrane can decrease apoB secretion by targeting it for degradation, while that not bound to ER membrane (called soluble receptor) can greatly increase apoB secretion (figure 6).

When VLDL enters into the bloodstream, TG-VLDL is hydrolyzed by lipoprotein lipase (LPL), which is located on the luminal side of capillaries and arteries, into free fatty acids, which are utilized by muscle and adipose tissue. After TG is lost, VLDL is converted into intermediate density lipoprotein (IDL) or low density lipoprotein (LDL). IDL and LDL are referred to as VLDL remnants, which are cleared from plasma greatly by the liver and partly by peripheral tissues through an apoE-mediated process. VLDL remnant clearance is one the important mechanisms for the homeostasis of plasma lipids (Brody, 1999).

In summary, hepatic VLDL assembly and secretion is a complex biochemical process, in which many factors are involved. The rate of VLDL-TG secretion depends on the availability of both lipid and apolipoprotein components. Factors which can rescue apoB from intracellular

degradation, such as MTP, LDR, apoE, are predicted to be able to increase VLDL-TG secretion. The contrast of hepatic VLDL secretion and VLDL remnant clearance from plasma is the determinant of lipid homeostasis in plasma.

POSSIBILITY OF GENE THERAPY FOR BOVINE FATTY LIVER

Gene therapy is defined as the intracellular delivery of genetic material to generate a therapeutic effect by correcting an existing abnormality or providing cells with a new function (Drew and Martin, 1999). The genetic materials considered for use are intended to replace a defective or missing gene, or to augment the functions of the genes present. Gene therapy offers the exciting potential of a new therapeutic approach for currently untreatable diseases.

The possibility of gene therapy for bovine fatty liver lies in the technology of gene therapy itself on the one hand, and the understanding of molecular mechanism of fatty liver development on the other. The first aspect looks more and more optimistic. Since the first trial of gene therapy for human adenosine deaminase deficiency in 1990, great progresses have been made to improve the effectiveness of gene therapy. Among the exciting progresses are the establishment of tissue-targeted gene delivery systems (Nishikawa, 2000), new approaches of long-term expressions of foreign genes *in vivo* (Zhang et al., 2000; Miao et al., 2000), the precise regulation technique of delivered gene expression *in vivo* (Ye, 1999). All these achievements make gene therapy more and more realistic. Up to now, over 100 protocols for human gene therapy have been established and approved for trials (Smith, 1999). Although there have been absolutely no approaches which are aimed to treat animal diseases, the strategy and the technological progress of human gene therapy are also applicable to animals.

Even though the mechanism of fatty liver development is not clearly known, the general understanding of VLDL metabolism provides a good basement for exploiting gene therapy for this disorder. The results of recent researches in transgenic animals are more realistic and encouraging evidences for the possibility of developing the approach of gene therapy for bovine fatty liver.

Tsukamoto et al. (2000) introduced human apoE genes into apoE-deficient mice and found apoE-expressed mice had 4-5-fold hepatic VLDL triglyceride production rate compared to apoE-deficient mice. Three isoforms of apoE, i.g. E2, E3 and E4 had similar extent of effects on VLDL triglyceride secretion (figure 7). In Mensenkamp et al. (1999)'s study, hepatic apoB secretion is not affected, but the size of VLDL particle increases in apoE-expressed mice. The *in vivo* VLDL-triglyceride production rate increase up

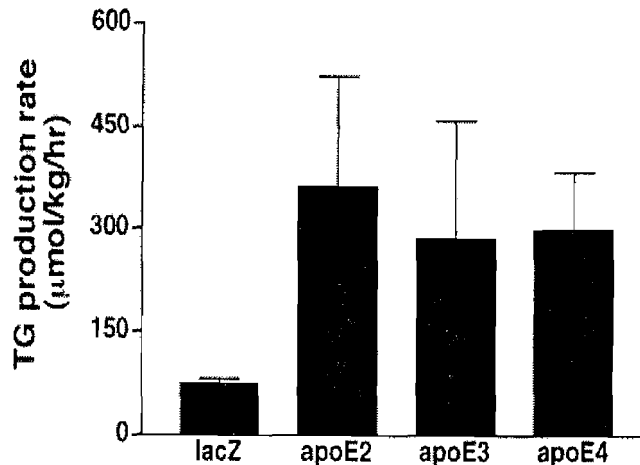


Figure 7. Hepatic TG production of apoE-deficient mice at 3 h after injection with Adh-apoE compared to LacZ (From Tsukamoto, 2000)

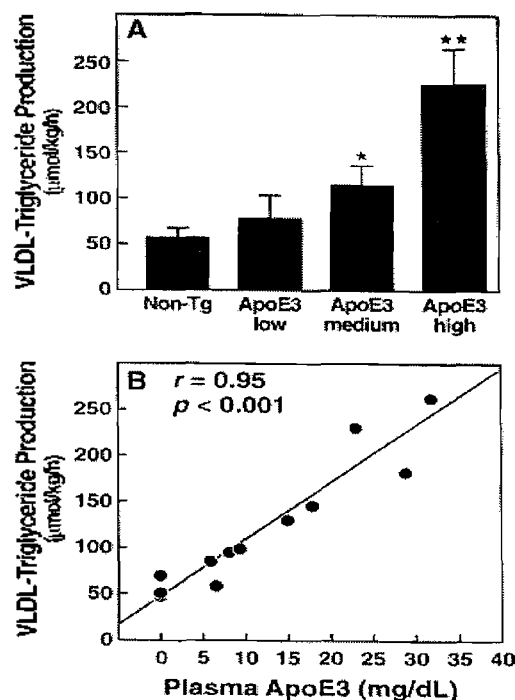


Figure 8. Effects of apoE3 expression level in rabbits on hepatic VLDL-TG production (A) and the correlation of VLDL-TG production with plasma apoE3 level (B)
* $p < 0.001$ vs nontransgenic; ** $p < 0.001$ vs apoE3 medium expresser. (From Huang, et al. 1999)

to 5-fold. These two studies are quite consistent and confirm the previous studies in mice (Huang et al., 1998; Kuipers et al., 1997, 1996). Huang et al. (1999) observed the stimulatory effect of apoE expression on hepatic VLDL production in transgenic rabbits and found a linear correlation of hepatic VLDL secretion to the expression level of apoE3 (figure 8). All the above researches indicate

Table 1. Hepatic concentrations (AU*/10⁶ cells) of apolipoprotein B (ApoB) and its mRNA in nonlactating and lactating cows

	Nonlactating		Lactating cows(n=8)			
	Pregnant (n=5)	Nonpregnant (n=4)	Wk1	Wk2	Wk4	Wk12
ApoB	35.8	33.4	24.0	26.0	27.5	30.2
mRNA						
SE	7.9	4.8	3.2	4.4	3.7	3.0
ApoB	81.3 ^A	76.2 ^A	25.1 ^{aB}	30.9 ^{aA}	35.0 ^{aA}	93.7 ^{ba}
SE	14.8	21.0	3.4	15.1	4.9	24.1

^{A,B}: Comparison between lactating cows by Mean-Whitney test (p<0.01)

^{a,b}: Comparison between different weeks of lactation by Student's test (p<0.01). * Arbitrary units. (From Gruffat, 1997)

that apoE-gene transfer is an efficient way to increase hepatic assembly and secretion of VLDL.

The success that apoE overexpression improves hepatic VLDL secretion in small mammalian animals, in combination with the current understanding of hepatic lipid metabolism in dairy cows lead to the reasonable assumption that apoE-gene transfer into transition cows may be the most effective strategy for alleviating fatty liver. Gruffat (1997) found that hepatic concentrations of apoB were lower in lactating cows at week 1, 2, and 4 postpartum compared to nonlactating cows, while apoB mRNA did not vary significantly among treatments (table 1). This result shows that apoB synthesis is subject to post-transcriptional regulation in dairy cows, as in other animals. Therefore, the proportion of apoB destined for VLDL assembly to that for intracellular degradation depends mainly on lipids and other proteins required for VLDL assembly. Although some researchers suggest that the availabilities of cholesterol or phospholipids be the limiting factor (Herdt, 1988; Katayama, 1994), their availabilities are usually sufficient to support VLDL secretion (Dixon and Ginsberg, 1993). Therefore, proteins like MTP and apoE are most likely to be the limiting factors. Tietge et al. (1999) found hepatic overexpression of MTP in wild mice resulted in almost 3-fold increase of hepatic triglyceride secretion and 74% higher of apoB secretion compared to control mice, indicating MTP is limiting at normal condition for mice. However, it may not be true for ruminants. Bremmer et al. (1998) failed to find the relationship between hepatic MTP activity and VLDL production or severity of fatty liver, demonstrating that MTP is not a key factor for low VLDL secretion. On the other hand, cows have low level of apoE expression (Brantmeier et al., 1988). Therefore, it is plausible that ectopic expression of apoE in transition cows could hopefully increase hepatic VLDL secretion and in turn alleviate the severity of fatty liver.

HURDLES OF GENE THERAPY FOR BOVINE FATTY LIVER

It can be expected that bovine fatty liver can be treated by gene therapy in the future. However, there are substantial hurdles for gene therapy to be a clinical reality. First hurdle is gene therapy itself. Although huge advances have been made toward developing technologies for clinical gene-therapy applications, gene therapy is still during its formative stage. How to deliver an interest gene precisely into the target organ (or cells) and to make the gene expressed for a sufficient period of time at a level enough to produce appropriate quantities of a biologically active protein to reverse the abnormalities without any side effects remains to be further studied (Smith, 1999; Blau and khavari, 1997).

Secondly, molecular mechanisms of liver metabolism and fatty liver development in cows are not clearly understood. Although there were a lot of studies on the physical and chemical characteristics of bovine plasma lipoproteins early in the 1980's (Forte, 1981; Grummer et al., 1983; Chapman, 1980) there were so far few studies on hepatic lipoprotein and apolipoprotein metabolism of cattle, especially the comparative studies of cattle to other animals such as mice and humans from which majority of current understandings on apolipoprotein metabolism come. Cautions should be taken when interpreting data from mice or humans to deal with bovine fatty liver. Compared to other animals, bovine liver may play a unique role in lipid metabolism, being compatible with its unique digestive physiology. The fact that dairy cows are prone to fatty liver during periparturient period itself may be an evidence. The limited information from previous literatures suggests that mature cattle may have some differences in plasma lipoprotein and apolipoprotein profiles from preruminant calves (Forte, 1981; Bauchart et al., 1989) and from other animals (Bauchart et al., 1989; Chapman, 1980). All the above facts indicate that there exist a lot of problems toward

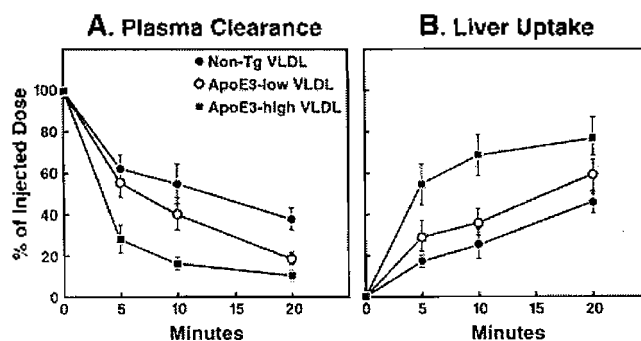


Figure 9. Effects of apoE3 expression in rabbits on plasma clearance and liver uptake of ¹²⁵I-VLDL. Tg: transgenic (From Huang et al., 1999)

the gene therapy for bovine fatty liver.

Even though apoE is the hopefully correct candidate protein for gene therapy, many problems remain to be solved. Biological effects of apoE overexpression depend on the expression levels and may vary with animal species. It is found in apoE-deficient mice that hepatic VLDL triglyceride secretion depended on the dose of apo E gene injected and there existed a certain threshold of gene introduced for significant VLDL production (Mensenkamp et al., 1999). If the same is true for dairy cattle, the amount of apoE gene required may be too large to be realistic for therapeutic purpose. Huang et al. (1999) found in transgenic rabbit that the expression level of apoE3 increased hepatic VLDL secretion and plasma VLDL clearance rate, impaired VLDL lipolysis, and delayed LDL clearance (figure 9). Plasma lipid concentrations rely on the balance of the four effects. High level expression of apoE would result in hypercholesterolemia and hyperlipidemia. If the same phenomenon occurs in dairy cows, then apoE expression level will be very important for curing fatty liver without adverse effects. However, differences between animal species should also be realized. Huang et al. (1998,1999) found that the similar level of apoE3 expression in the mice and rabbit resulted in different extent of stimulating VLDL production. The stimulation was much higher in rabbit than in mice. It is not known whether and how cows are different at this point. Therefore, optimizing apoE level is an essential and hard task for developing gene therapy for bovine fatty liver.

The existence of many hurdles indicates that gene therapy for bovine fatty liver has a long way to go for becoming a practical therapeutical tool. However, gene therapy can also be applied now for studying mechanisms of fatty liver development rather than for clinical therapy. Problems will be solved eventually by means of the applications of technologies from nutritional science and molecular biology.

CONCLUSION

The mechanisms by which bovine fatty liver develops are complex and not yet well known. The current understandings of hepatic VLDL metabolism and the application of molecular biology offer the possibility to alleviate fatty liver by gene therapy. This possibility lies in the introduction of apoE gene to stimulate hepatic VLDL secretion. Lack of any studies on apoE gene transfer in ruminants means that a lot of issues need to be characterized before gene therapy becomes a clinical tool for alleviating fatty liver. However, the technologies for gene therapy can be applied now for studying and solving the current puzzles. Bovine fatty liver will be clearly understood and finally be treatable by adopting multi-

disciplinary knowledges and technologies.

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