Original Article

Gene expressions of de novo hepatic lipogenesis in feline hepatic lipidosis

Chiara Valtolina[1] (ID), Joris H Robben[1], Monique E van Wolferen[1], Hedwig S Kruitwagen[1], Ronald J Corbee[1], Robert P Favier[2] and Louis C Penning[1]

Abstract

Objectives The aim of this study was to evaluate if de novo hepatic lipid synthesis contributes to fatty acid overload in the liver of cats with feline hepatic lipidosis (FHL).

Methods Lipogenic gene expression of peroxisome proliferator-activated receptor-alpha (*PPAR-α*), peroxisome proliferator-activated receptor-gamma (*PPAR-γ*), fatty acid synthase (*FASN*) and sterol regulatory element-binding factor (*SREBF1*) were evaluated using quantitative RT-PCR in liver tissue of six cats with FHL and compared with the liver tissue of eight healthy cats.

Results In liver tissue, *PPAR-α, PPAR-γ* and *FASN* mRNA expression levels were not significantly different ($P > 0.12$, $P > 0.89$ and $P > 0.5$, respectively) in the FHL group compared with the control group. *SREBF1* gene expression was downregulated around 10-fold in the FHL group vs the control group ($P = 0.039$).

Conclusions and relevance The downregulation of *SREBF1* in the liver tissue of cats with FHL does not support the hypothesis that de novo lipogenesis in the liver is an important pathway of fatty acid accumulation in FHL.

Keywords: Feline hepatic lipidosis; de novo lipogenesis; *SREBP-1c*; qRT-PCR

Introduction

Feline hepatic lipidosis (FHL) is considered the most common hepatobiliary disease in cats and is characterised by the accumulation of excessive triglycerides (TGs) in more than 80% of hepatocytes, resulting in liver dysfunction and cholestasis.[1–4] The pathophysiology of FHL is complex and the recent literature has highlighted how FHL results from multiple alterations in the pathways involved in the hepatic uptake of fatty acids (FAs), lipoproteins and glucose, TG degradation and FA oxidation, and lipoprotein secretion in the form of very-low-density lipoproteins.[1,3–5] De novo TG synthesis in the liver could cause some of the hepatic FA load and may further exacerbate liver fat accumulation.[4–6]

De novo lipogenesis (DNL) is a complex and highly regulated metabolic pathway.[7,8] In normal conditions DNL converts excess carbohydrate into FAs, which are then esterified to storage triacylglycerols. The main product of DNL is palmitic acid (C16:0). Palmitic acid can be desaturated by stearoyl-CoA desaturase 1 (SCD-1) to produce palmitoleic acid, or it can be elongated to yield stearic acid (C18:0) and other shorter FAs.[8–10]

In healthy humans, DNL is active in both liver and adipose tissue, whereas in healthy cats the adipose tissue is primarily involved and the liver plays a secondary role. Furthermore, while glucose is the precursor for DNL in humans, in cats the substrate to form the FA palmitate is acetate.[3,11–13] DNL is considered to be a minor contributor to lipid formation and lipid homeostasis in healthy individuals.[14–17]

However, in humans, hepatic DNL has been demonstrated to contribute to liver steatosis in different

[1]Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands
[2]Evidensia Netherlands, Vleuten, The Netherlands

Corresponding author:
Chiara Valtolina DVM, Dipl ACVECC, Dipl ECVECC, Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 108, Utrecht, 3584 CM, The Netherlands
Email: c.valtolina@uu.nl

metabolic diseases that cause deregulation of the lipogenic pathways, such as obesity, type 2 diabetes and non-alcoholic fatty liver disease (NAFLD).[18–22]

The adipogenic transformation of hepatocytes, with the expression of gene profiles characteristic of healthy adipose tissue, contributes to liver overload with excess free FAs (FFAs).[8,23,24] DNL has been demonstrated to contribute up to 26% of FFAs that accumulate in the liver of patients with NAFLD.[8,10,25]

The role of the molecular mediators of lipogenesis in NAFLD has recently been reviewed.[26]

Sterol regulatory element-binding protein 1c (*SREBP-1c*) has been defined as the pivotal controller of lipid biosynthesis and main regulator of the expression of several genes involved in lipogenesis.[27–30] In patients with NAFLD and obese patients, elevated de novo hepatic FA synthesis and secondary TG accumulation in the liver has been reported to be secondary to increased mRNA expression of hepatic *SREBP-1c*. Increasing *SREBP-1c* by inducing lipogenic gene expression, such as those for fatty acid synthase (*FASN*) and peroxisome proliferator-activated receptor gamma (*PPAR-γ*), and suppressing the mRNA expression of peroxisome proliferator-activated receptor alpha (*PPAR-α*), favours hepatic lipogenesis over FA oxidation and leads to steatosis.[28–33] *FASN* is involved in conversion of malonyl-CoA into 16-carbon saturated FA palmitate.[26] *PPAR-γ* is required for normal adipocyte differentiation and is expressed at very low levels in the liver of healthy individuals. Overexpression of *PPAR-γ* in the liver leads to hepatic steatosis, also by enhancing the expression of other adipogenic genes in the liver.[32,34–36]

The physiological significance of DNL in NAFLD has been recently reviewed by Hodson and Frayn.[27] They argued that the most important role of DNL might not be its quantitative contribution to hepatic FA overload, but its part in the suppression of FA oxidation.[27] Malonyl-CoA, a key a metabolic intermediate in de novo FA biosynthesis in lipogenic tissues, is, in fact, a potent inhibitor of the mitochondrial outer membrane enzyme carnitine-palmitoyl transferase 1 (CPT-1).[37] CPT-1 is responsible for the transport of fatty acyl-CoA into the mitochondrion, a fundamental step in FA oxidation.[38] The increased expression of *SREBP-1c* in NAFLD suppresses *PPAR-α*, further inhibiting CPT-1 and FA beta-oxidation.[31]

The role of DNL in the development of hepatic lipidosis has been evaluated in the American mink (*Neovison vison*), an obligated carnivore often used as an experimental animal model for NAFLD.[12,13] These animals share physiological similarities with the domestic cat and they can easily develop hepatic lipidosis after a short period of anorexia.[4,5,13,39] In a recent study evaluating mRNA expression of lipogenic genes in the liver of mink with hepatic lipidosis, Rouvinen-Watt et al confirmed that liver steatosis is partially secondary to the activation of the hepatic DNL.[13]

The concept that hepatic DNL could contribute to liver FA overload in cats with hepatic lipidosis was, for the first time, introduced by Hall et al in their study on the lipid composition of hepatic and adipose tissue in cats with FHL.[40] The FA composition of visceral adipose tissue and of the liver of cats with HL revealed greater percentages of palmitate and monounsaturated FAs (MUFAs) than in healthy controls. The increased hepatic concentrations of palmitate in cats with HL vs healthy cats supported the hypothesis that some de novo synthesis of FAs occurs both in the liver and adipose tissue of cats with hepatic lipidosis.[40] Furthermore, in FHL, acetate, like other ketone bodies, is increased as a result of a more complex catabolic state that includes increased insulin resistance and decreased tolerance to glucose.[5,11,41] The increased acetate in the liver of cats with FHL constitutes a potential excess substrate for hepatic DNL, contributing and worsening hepatic FA overload.

To date, the concept of adipogenic transformation of hepatocytes and its contribution to hepatic FA accumulation has not been adequately evaluated in cats.

The aim of the present study was to evaluate adipogenic transformation of hepatocytes and de novo lipid synthesis by determination of lipogenic gene expression of *PPAR-α, PPAR-γ, FASN* and *SREBF1* (equivalent in function to *SREBP-1c*) using quantitative RT-PCR (qRT-PCR) in the liver tissue of cats with FHL.

Materials and methods

This study was approved by the Committee for the Ethical Care of Animals of Utrecht University. Written, informed consent was obtained from all cat owners prior to study enrolment of the cats.

Animals

Cats admitted from January 2014 to January 2015 to the Department of Clinical Science of Companion Animals (DCSCA) of the Faculty of Veterinary Medicine, Utrecht University diagnosed with HL based on clinical signs (anorexia, vomiting and icterus), ultrasonography findings (diffuse echodensity of the liver) and cytological evaluation of fine-needle aspirates of the liver consistent with FHL were considered for the study. Only cats that were euthanased owing to deterioration of their clinical condition, despite intense treatment and/or because of financial constraints, were enrolled in the study.

In cats with FHL, wedge liver biopsies were immediately collected post mortem via laparotomy. The biopsies were rinsed in normal saline (NaCl 0.9%), rapidly frozen in liquid nitrogen and then stored at −80°C. These frozen biopsy specimens were used to perform qRT-PCR.

Table 1 Primers for *FASN, PPAR-γ, SREBF1* and *PPAR-α*

Gene	Primer 5′ → 3′	Annealing temperature (°C)	Amplicon size (bp)
FASN	F: GAAATCGGCAAATTCGACCT R: CTGTTCCCACCTTCATCCA	65	115
PPAR-γ	F: TGTGACCTTAACTGTCGTATCC R: CTTCTCTTTCTCCGCCTGTG	66–67	134
SREBF1	F: CGTTTCTTCGTGGATGGG R: ACAAATTCAGTGCTGCTC	63	140
PPAR-α	F: GACAAATGTGACCGTAGCTG R: AAACGAATTGCGTTATGGGA	60	109
RPS5	F: CAGGTCTTGGTGAATGCG R: CCAGATGGCCTGATTCAC	58	129
GUSB	F: TGACATCACCATCAGCACCAGC R: GCCTTCCTCATCCAGAAGACGC	67	114
YWHAZ	F:GAAGAGTCCTACAAAGACAGCACGC R: AATTTTCCCCTCCTTCTCCTGC	65	115

Sequences based on Kruitwagen et al and Penning et al[44,45]
F = forward; R = reverse

Aged-matched liver biopsies of eight healthy cats were used as controls. These liver biopsies were retrieved from surplus material from a study on the effect of administration of vitamin A on skeletal and hepatic tissue as part of the university's 3R-policy.[42] Good health in these cats was based on a normal physical examination and haematological, biochemical and coagulation parameters within the reference intervals. In this study, percutaneous ultrasound-guided liver biopsies at the beginning and end of the study were taken with a 16 G, 20 cm long biopsy needle (Pro-Mag Biopsy Needle; Argon Medical Devices) under general anaesthesia. The liver biopsies were snap frozen in liquid nitrogen and then stored at −80°C. Only liver biopsies taken at the beginning of the study before supplementation of vitamin A was initiated were used for evaluation of lipogenesis gene expression.

RNA isolation, reverse transcription and quantitative RT-PCR
Total RNA was isolated from liver tissue using a RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. An on-column DNase-I (QIAFEB, RNase-free DNase kit) treatment was included to avoid contamination with residual genomic DNA. RNA concentrations and quality were measured spectrophotometrically using the Nanodrop ND-1000 (Isogen Life Science). RNA integrity was checked on a Bioanalyzer 2100 (Agilent Technologies). The RNA integrity number of all samples exceeded seven. Per sample, at least 3 µg of RNA was used for further processing. cDNA was synthesised from all RNA samples with the iScriptTM cDNA Synthesis Kit (BioRad), which contained both oligo-dT and random hexamer primers to ensure that the cDNA levels were reliably representative of the mRNA levels.

Around 600 ng of RNA was incubated with iScript reaction mix, iScript reverse transcriptase and nuclease-free water at 42°C for 30 mins in a 60 µl reaction volume.

As required under MIQE-precise expression stability of the reference genes was evaluated and a combination of ribosomal protein S5 (*RPS5*), β-glucuronidase (*GUSB*) and tyrosine 3-monooxygenase/tryptophan 5-momooxygenase activation protein zeta isoform (*YWHAZ*) was optimal.[43] Primer sequences for specific sequence-confirmed amplicons and optimal annealing temperature are described in Table 1. The qRT-PCRs were performed in duplicate using the BioRad detection system. Amplifications were carried out in a volume of 25 µl containing 12.5 µl 2 × SYBR green supermix (BioRad), 0.4 µM forward and reverse primer, and 1 µl cDNA and Milli-Q water. Cycling conditions were as follows: denaturation at 95°C for 3 mins, followed by 45 cycles of denaturation (95°C for 10 s) and amplicon-specific annealing temperature for 30 s. A melt curve analysis was performed for every reaction to verify amplicon specificity. IQ5 Real-Time PCR detection system software (BioRad) was used for data analysis. A no template control was also run in duplicate with each plate as a negative control. Expression levels were calculated taken the PCR efficiency into account; the PCR efficiency was always between 96% and 106%.

Data are reported as median (range). The Mann–Whitney U-test was used to determine significance for qRT-PCR results between groups, with statistical significance being set at $P < 0.05$.

Results
Animals
The FHL group consisted of six client-owned cats (four male castrated, one male intact and one female spayed)
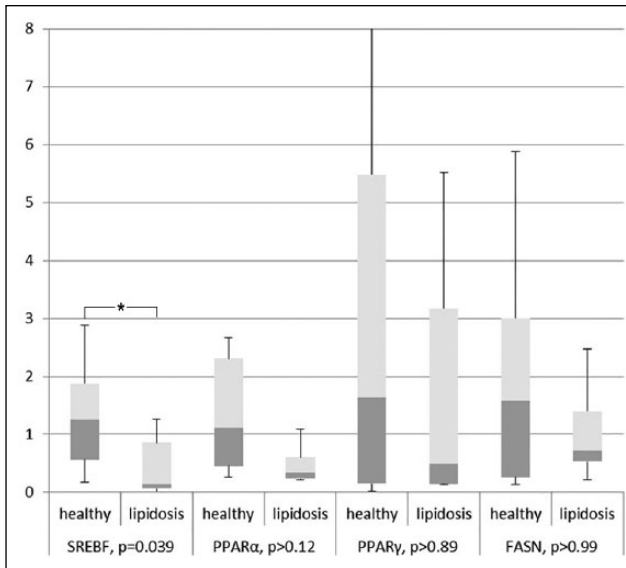
Figure 1 Presented are median, first quartile (box) and range (whiskers). Expression per gene is related to the average of the healthy control group, which is set at '1'. Expression corrected for three reference genes *RPS5, GUSB* and *YWHAZ*. *Only the expression of *SREBF1* is significantly downregulated in feline hepatic lipidosis samples compared with heathy controls

that met the inclusion criteria for the study. Three cats were European Shorthairs, two were Maine Coons, one was a Norwegian Forest Cat and one a Turkish Angora. Median age was 4 years and 4 months (range 2–8.5 years) and median body weight was 4 kg (range 3–6.7 kg). Anorexia, lethargy and icterus were the major problems for which 5/6 cats were referred to the DCSCA. One cat was admitted for dyspnoea secondary to chronic traumatic diaphragmatic rupture and developed HL while in hospital.

The control group consisted of six castrated males and two intact males cats. They all were European Shorthair cats. Median age was 4 years and 3 months (range 1.5–7 years) and median body weight was 4.6 kg (range 3–6.3 kg).

Lipogenic gene expression
In liver tissue, *PPAR-α, PPAR-γ* and *FASN* mRNA expression levels were not significantly different ($P >0.12$, $P >0.89$ and $P >0.5$, respectively) in the FHL group compared with the control group (Figure 1). *SREBF1* gene expression was downregulated around 10-fold in the FHL group vs the control group ($P = 0.039$) (Figure 1).

Discussion
In humans, in situations of metabolic dysregulation, DNL has been suggested to be abnormally increased and contributes to the pathogenesis of liver steatosis in diseases such as obesity and NAFLD.[10,20–22] The increased

DNL is characterised by the presence of the FA palmitate, the primary product of de novo FA synthesis, and the MUFA palmitoleic acid, synthesised from palmitate by the action of the enzyme SCD-1.[9,10]

In cats with FHL, a higher concentration of palmitate and MUFAs were found both in the visceral adipose tissue and liver, when compared with healthy cats.[40] This finding was suggestive of an increased DNL in visceral adipose tissue and most likely also in the liver as part of the pathophysiological mechanism of liver steatosis, contributing and worsening FA liver overload.[40]

In this study the decrease in liver *SREBF1* gene expression and no expression change in other DNL-related enzymes suggest that DNL does not occur in the liver of cats with FHL. This is in contrast with what has been reported in humans with NAFLD and in other obligated carnivores.[13,18–21] Furthermore, expression of *PPAR-α* was similar to its expression in healthy cats. This suggests that *CPT-1a* expression and the following degree of β-oxidation in the liver of cats with hepatic lipidosis is not affected by a downregulation of *PPAR-α.*

Using labelled FAs, Donnelly et al concluded that 59% of TGs in the livers of patients with NAFLD were from increased lipolysis in the adipose tissue and increased FA flux to the liver, 26% were from hepatic DNL and 15% were from the diet.[25] Hepatic DNL contributes significantly to FA accumulation in the liver of subject with NAFLD.

The development of hepatic lipidosis has been investigated in the American mink, as a model of NAFLD.[12,13] In the American mink, food deprivation for 3 days was adequate to induce hepatic lipidosis, with liver fat percentage increasing twice in the fasted group vs the control group. The liver steatosis in the initial days of fasting was secondary to the flux of FAs from extensive peripheral lipolysis from the visceral adipose tissue.[12,13] In the following days, the percentage of liver lipid accumulation increased dramatically to the end of the 7-day fasting period. Significant increases were observed in the levels of mRNA encoding for acetyl-CoA carboxylase 1 (*ACC-1*) and *FASN* after 5–7 days of fasting compared with non-fasted mink. It was concluded that hepatic DNL could have further exacerbated liver fat accumulation in fasted minks and that 5–7 days of fasting appeared to be the critical time period for this to develop. Although *SREBP-1c* was not measured in this study, the higher expression of *FASN* and *ACC-1*, and the higher hepatic palmitate and MUFA concentrations, suggested that the gene expression of the key enzymes for DNL was increased. Feeding the minks for 28 days after the end of the fasting period allowed the relative expression of *ACC-1* and *FASN* to return to pre-fasting levels.[13]

In a model of a diet-induced overweight state in cats, lipogenic gene expression (*SREBP-1c, FASN* and ATP citrate lyase [*ACLY*]) in lipid sensitive tissue, that is liver,

subcutaneous and abdominal adipose tissue, has been evaluated and compared with lean animals.[46] As already demonstrated in humans, a positive energy imbalance due to overnutrition and obesity can activate *SREBP-1c* and other secondary lipogenic gene expressions, enhancing lipogenesis in adipose and liver tissues contributing to liver steatosis.[18,19,21] In the overweight cats, mRNA expression of *SREBP-1c* and ATP citrate lyase were markedly decreased in liver tissue, whereas *FASN* expression remained similar to that of control cats. Abdominal *SREBP-1c* mRNA expression was markedly lower in abdominal subcutaneous and higher in omental adipose tissues of overweight cats than in control cats. The conclusion of this study was that in an overweight cat model DNL does not occur in the liver, but it occurs in the visceral adipose tissue.[46] This supports the finding of Hall et al,[40] that the origin of the enhanced hepatic TGs in cats with FHL is the visceral adipose tissue and not the liver.

Our study has some limitations. Although the collection process of healthy liver tissue (ie, needle biopsies) and of FHL livers (ie, post-mortem wedge material) were different, there are no indications that the biopsy technique itself could have caused differences in relative mRNA expression levels.

In order to validate the qRT-PCR data, the reduced mRNA level needs to be confirmed by quantitative measurement at the protein level. This confirmation step is currently hampered by the very low hepatic expression of *SREBF1* together with the lack of validated antibodies for *SREBF1* in feline tissues. Interestingly, the downregulation of *SREBF1*, as observed here in vivo, is similar to the downregulation observed in three-dimensional feline liver organoids cultured in vitro under FA overload, lending credence to the robustness of the qRT-PCR measurements.[44]

Conclusions

The downregulation of *SREBF1* in the liver tissue of cats with FHL does not support the hypothesis that DNL in the liver is an important pathway of FA accumulation in FHL. As cats with hepatic lipidosis have an increased concentration of palmitate and MUFAs vs healthy cats in their liver and visceral adipose tissue, DNL in FHL most likely occurs in the abdominal adipose tissue. Subsequently, the de novo synthesised MUFAs are transported from the adipose tissue to the liver, where they contribute to fat overload in FHL.

ORCID iD Chiara Valtolina https://orcid.org/0000-0002-4601-8885

References

1 Armstrong PJ and Blanchard G. **Hepatic lipidosis in cats.** *Vet Clin North Am Small Anim Pract* 2009; 39: 599–616.

2 Center SA, Guida L, Zanelli MJ, et al. **Ultrastructural hepatocellular features associated with severe hepatic lipidosis in cats.** *Am J Vet Res* 1993; 54: 724–731.

3 Center SA. **Feline hepatic lipidosis.** *Vet Clin North Am Small Anim Pract* 2005; 35: 225–269.

4 Valtolina C and Favier RP. **Feline hepatic lipidosis.** *Vet Clin North Am Small Anim Pract* 2017; 47: 683–702.

5 Verbrugghe A and Bakovic M. **Peculiarities of one-carbon metabolism in the strict carnivorous cat and the role in feline hepatic lipidosis.** *Nutrients* 2013; 5: 2811–2835.

6 Jiang ZG, Robson SC and Yao Z. **Lipoprotein metabolism in nonalcoholic fatty liver disease.** *J Biomed Res* 2013; 27: 1–13. DOI: 10.7555/JBR.27.20120077.

7 Reddy JK and Rao MS. **Lipid metabolism and liver inflammation. II. Fatty liver disease and fatty acid oxidation.** *Am J Physiol Gastrointest Liver Physiol* 2006; 290: G852–8. DOI: 10.1152/ajpgi.00521.2005.

8 Ameer F, Scandiuzzi L, Hasnain S, et al. **De novo lipogenesis in health and disease.** *Metabolism* 2014; 63: 895–902.

9 Lee JJ, Lambert JE, Hovhannisyan Y, et al. **Palmitoleic acid is elevated in fatty liver disease and reflects hepatic lipogenesis.** *Am J Clin Nutr* 2015; 101: 34–43.

10 Lambert JE, Ramos-Roman MA, Browning JD, et al. **Increased de novo lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease.** *Gastroenterology* 2014; 146: 726–735.

11 Aroch I, Shechter-Polak M and Segev G. **A retrospective study of serum beta-hydroxybutyric acid in 215 ill cats: clinical signs, laboratory findings and diagnoses.** *Vet J* 2012; 191: 240–245.

12 Rouvinen-Watt K, Mustonen AM, Conway R, et al. **Rapid development of fasting-induced hepatic lipidosis in the American mink (*Neovison vison*): effects of food deprivation and re-alimentation on body fat depots, tissue fatty acid profiles, hematology and endocrinology.** *Lipids* 2010; 45: 111–128.

13 Rouvinen-Watt K, Harris L, Dick M, et al. **Role of hepatic de novo lipogenesis in the development of fasting-induced fatty liver in the American mink (*Neovison vison*).** *Br J Nutr* 2012; 108: 1360–1370.

14 Richard MJ, Holck JT and Beitz DC. **Lipogenesis in liver and adipose tissue of the domestic cat (*Felis domestica*).** *Comp Biochem Physiol B* 1989; 93: 561–564.

15 Campos CF, Duarte MS, Guimaraes SE, et al. **Review: animal model and the current understanding of molecule dynamics of adipogenesis.** *Animal* 2016; 10: 927–932.

16 Bergen WG and Mersmann HJ. **Comparative aspects of lipid metabolism: impact on contemporary research and use of animal models.** *J Nutr* 2005; 135: 2499–2502.

17 Bjorntorp P and Sjostrom L. **Carbohydrate storage in man: speculations and some quantitative considerations.** *Metabolism* 1978; 27 Suppl 2: 1853–1865.

18 Strable MS and Ntambi JM. **Genetic control of de novo lipogenesis: role in diet-induced obesity.** *Crit Rev Biochem Mol Biol* 2010; 45: 199–214.

19 Byrne CD. **Fatty liver: role of inflammation and fatty acid nutrition.** *Prostaglandins Leukot Essent Fatty Acids* 2010; 82: 265–271.

20 Choudhury J and Sanyal AJ. **Insulin resistance and the pathogenesis of nonalcoholic fatty liver disease.** *Clin Liver Dis* 2004; 8: 575–594.

21 Diraison F, Dusserre E, Vidal H, et al. **Increased hepatic lipogenesis but decreased expression of lipogenic gene in adipose tissue in human obesity.** *Am J Physiol Endocrinol Metab* 2002; 282: E46–E51.

22 Rector RS, Thyfault JP, Wei Y, et al. **Non-alcoholic fatty liver disease and the metabolic syndrome: an update.** *World J Gastroenterol* 2008; 14: 185–192.

23 Pardina E, Baena-Fustegueras JA, Llamas R, et al. **Lipoprotein lipase expression in livers of morbidly obese patients could be responsible for liver steatosis.** *Obes Surg* 2009; 19: 608–616.

24 Westerbacka J, Kolak M, Kiviluoto T, et al. **Genes involved in fatty acid partitioning and binding, lipolysis, monocyte/macrophage recruitment, and inflammation are over-expressed in the human fatty liver of insulin-resistant subjects.** *Diabetes* 2007; 56: 2759–2765.

25 Donnelly KL, Smith CI, Schwarzenberg SJ, et al. **Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease.** *J Clin Invest* 2005; 115: 1343–1351.

26 Browning JD and Horton JD. **Molecular mediators of hepatic steatosis and liver injury.** *J Clin Invest* 2004; 114: 147–152.

27 Hodson L and Frayn KN. **Hepatic fatty acid partitioning.** *Curr Opin Lipidol* 2011; 22: 216–224.

28 Horton JD, Goldstein JL and Brown MS. **SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver.** *J Clin Invest* 2002; 109: 1125–1131.

29 Horton JD, Goldstein JL and Brown MS. **SREBPs: transcriptional mediators of lipid homeostasis.** *Cold Spring Harb Symp Quant Biol* 2002; 67: 491–498.

30 Horton JD. **Sterol regulatory element-binding proteins: transcriptional activators of lipid synthesis.** *Biochem Soc Trans* 2002; 30: 1091–1095.

31 Pettinelli P, Del Pozo T, Araya J, et al. **Enhancement in liver SREBP-1c/PPAR-alpha ratio and steatosis in obese patients: correlations with insulin resistance and n-3 long-chain polyunsaturated fatty acid depletion.** *Biochim Biophys Acta* 2009; 1792: 1080–1086.

32 Pettinelli P and Videla LA. **Up-regulation of PPAR-gamma mRNA expression in the liver of obese patients: an additional reinforcing lipogenic mechanism to SREBP-1c induction.** *J Clin Endocrinol Metab* 2011; 96: 1424–1430.

33 Yang ZX, Shen W and Sun H. **Effects of nuclear receptor FXR on the regulation of liver lipid metabolism in patients with non-alcoholic fatty liver disease.** *Hepatol Int* 2010; 4: 741–748.

34 Matsusue K, Haluzik M, Lambert G, et al. **Liver-specific disruption of PPARgamma in leptin-deficient mice improves fatty liver but aggravates diabetic phenotypes.** *J Clin Invest* 2003; 111: 737–747.

35 Yu S, Matsusue K, Kashireddy P, et al. **Adipocyte-specific gene expression and adipogenic steatosis in the mouse liver due to peroxisome proliferator-activated receptor gamma1 (PPARgamma1) overexpression.** *J Biol Chem* 2003; 278: 498–505.

36 Boelsterli UA and Bedoucha M. **Toxicological consequences of altered peroxisome proliferator-activated receptor gamma (PPARgamma) expression in the liver: insights from models of obesity and type 2 diabetes.** *Biochem Pharmacol* 2002; 63: 1–10.

37 Saggerson D. **Malonyl-CoA, a key signaling molecule in mammalian cells.** *Annu Rev Nutr* 2008; 28: 253–272.

38 Reddy JK and Hashimoto T. **Peroxisomal beta-oxidation and peroxisome proliferator-activated receptor alpha: an adaptive metabolic system.** *Annu Rev Nutr* 2001; 21: 193–230.

39 Eisert R. **Hypercarnivory and the brain: protein requirements of cats reconsidered.** *J Comp Physiol B* 2011; 181: 1–17.

40 Hall JA, Barstad LA and Connor WE. **Lipid composition of hepatic and adipose tissues from normal cats and from cats with idiopathic hepatic lipidosis.** *J Vet Intern Med* 1997; 11: 238–242.

41 Chan DL and Freeman LM. **Nutrition in critical illness.** *Vet Clin North Am Small Anim Pract* 2006; 36: 1225–1241.

42 Corbee RJ, Tryfonidou MA, Grinwis GC, et al. **Skeletal and hepatic changes induced by chronic vitamin A supplementation in cats.** *Vet J* 2014; 202: 503–509.

43 Bustin S and Penning LC. **Improving the analysis of quantitative PCR data in veterinary research.** *Vet J* 2012; 191: 279–281.

44 Kruitwagen HS, Oosterhoff LA, Vernooij IGWH, et al. **Long-term adult feline liver organoid cultures for disease modeling of hepatic steatosis.** *Stem Cell Rep* 2017; 8: 822–830.

45 Penning LC, Vrieling HE, Brinkhof B, et al. **A validation of 10 feline reference genes for gene expression measurements in snap-frozen tissues.** *Vet Immunol Immunopathol* 2007; 120: 212–222.

46 Lee P, Mori A, Takemitsu H, et al. **Lipogenic gene expression in abdominal adipose and liver tissues of diet-induced overweight cats.** *Vet J* 2011; 190: e150-3.