Hyperlipidemic Guinea Pig Model: Mechanisms of Triglyceride Metabolism Disorder and Comparison to Rat

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Guinea pigs and rats are both common animal models for hyperlipidemia studies. However, many recent studies have suggested that rats do not develop hypertriglyceridemia in response to cholesterol feeding. In the present work, the differences in triglyceride metabolism between guinea pigs and rats were investigated. Feeding a high-fat diet containing 0.1% cholesterol and 10% lard for 4 weeks led to a significant increase in plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG) and free fatty acid (FFA) in guinea pigs but not in rats. By contrast, hepatic TG levels in rats were greatly increased in response to the high-fat diet, while it remained unchanged in guinea pigs. Furthermore, the hepatic acyl CoA:diacylglycerol acyltransferase (DGAT) activity and microsomal triglyceride transfer protein (MTTP) mRNA levels in guinea pigs fed a high-fat diet were significantly higher than those in the control group, which implies an increased very-low-density lipoprotein (VLDL)-TG secretion rate in guinea pigs in response to a high-fat diet. Hepatic carnitine palmitoyltransferase-1 (CPT-1) activity and peroxisome proliferator-activated receptor-α (PPARα) mRNA levels were upregulated in guinea pigs, but not rats, fed a high-fat diet. These findings may explain the differences in plasma and hepatic TG concentrations between guinea pigs and rats. These results suggest that there are differences in triglyceride metabolism between the two species when fed high-fat diets.

Key words guinea pig; rat; triglyceride; very-low-density lipoprotein-triglyceride secretion; fatty acid β-oxidation

Hyperlipidemia is an important risk factor for coronary heart disease. A proper animal model plays vital roles in the researches on mechanisms of lipid disorder. The advantages of using guinea pigs for cholesterol and lipoprotein metabolism investigations have been elucidated in many studies. The guinea pig is a suitable animal model for studying hyperlipidemia because of its similarities to humans in transporting the majority of its circulating cholesterol in low-density lipoprotein (LDL), exhibiting moderate rates of hepatic cholesterol synthesis and catabolism, having higher concentrations of free compared to esterified cholesterol in the liver and in many other aspects of lipid metabolism.[1—5] To date, however, little attention has been paid to triglyceride metabolism in guinea pigs.

Rats have commonly been used for hyperlipidemia studies in the past, although their use has recently been in decline. They transport most of their serum cholesterol in the high-density lipoprotein (HDL) fraction, have higher ability of plasma cholesterol clearance and couldn't develope a hypertriglyceridemia response to cholesterol feeding. More and more researchers indicated that the mechanisms by which diet interventions and drug treatments alter plasma lipids and lipoprotein metabolism in rats are different from humans.[6—9]

In our previous studies, we also found that a high-fat diet containing 0.1% cholesterol and 10% lard induced typical hyperlipidemia and hypertriglyceridemia in guinea pigs but not in rats.[10] And there is less number of good hypertriglyceridemic models. Therefore, it is very meaningful to further research the mechanisms of triglyceride metablism disorder in guinea pigs.

The present study was designed to determine the comparative plasma and hepatic lipid responses of guinea pigs and rats to high-fat diets containing 0.1% cholesterol and 10% lard and to compare the enzyme activities and gene expression of molecules that closely related to triglyceride metabolism.

MATERIALS AND METHODS

Reagents Acetyl-CoA, Triton WR-1339, malonyl-CoA, nicotinamide adenine dinucleotide phosphate (NADPH), DL-dithiothreitol (DTT), 5,5′-dithiobis(2-nitrobenzoate) (DTNB), palmitoyl-CoA, carnitine, 1,2-dipalmitoyl-*sn*-glycerol, palmitoyl-CoA lithium salt and glyceryl tripalmitate were purchased from Sigma-Aldrich. The solvents (chloroform, hexane, 2-propanol and methanol) were of HPLC grade (J. T. Baker, U.S.A.). All other reagents were of analytical grade and purchased from the Beijing Chemicals Company. The triglyceride (TG), total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) assay kits were purchased from BioSino Bio-technology and Science, Inc. The lipoprotein lipase (LPL) and free fatty acids (FFA) kits were obtained from the Nanjing Jiancheng Bioengineering Institute. The RNApure high-purity total RNA rapid extraction kit, Super reverse transcription (RT) Kit (First Strand cDNA Synthesis Kit) and 2×SYBR real-time polymerase chain reaction (PCR) premixture were purchased from the BioTeke Corp. The primers for microsomal triglyceride transfer protein (MTTP) and peroxisome proliferator-activated receptor-α (PPARα) were synthesised by the Shanghai Sangon Biological Engineering Technology and Services Co., Ltd., China.

Diets and Animals The diets were designed to meet the nutritional requirements of guinea pigs and rats and were prepared by the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences and Peking Union Medical College. The high-fat diets were similar to the control diet but were supplemented with 10% lard and 0.1% cholesterol, as shown in Table 1.

Forty Hartley guinea pigs, all male and weighing 200—

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Table 1. Composition of Experimental Diets

Component	Control diets (g/100 g)	High-fat diets (g/100 g)
Protein	19.3	19.3
Fat mix	3.2	3.2
Mineral mix[a)]	8.2	8.2
Vitamin mix[a)]	1.1	1.1
Fibre	11.8	11.8
Cholesterol[b)]	0	0.1
Lard	0	10

a) Mineral and vitamin mix adjusted to meet National Research Council requirements for guinea pigs and rats. *b*) Dietary cholesterol was 0.1 g/100 g for guinea pig and rat models.

250 g, were randomly assigned to two groups: a control group and a model group. Thirty-two Wistar rats, all male and weighing 180—200 g, were also assigned to two groups: a control group and a model group. Guinea pigs and rats in the control groups were fed control diets, while animals of both model groups were fed diets containing 10% lard and 0.1% cholesterol for 4 weeks. All the animals were housed in a temperature-controlled room (24 °C) with a 12-h light and dark cycle and had free access to food and water.

All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996, and the experimental procedures were approved by the Institutional Animal Care and Welfare Committee of Institute of Medicinal Plant Development.

Sample Collection and Processing At the end of the experiment (4 weeks), half of the animals were used to detect the very-low-density lipoprotein (VLDL)-TG secretion rate; the remainder were food-deprived and anesthetised with urethane (1 g/kg). Blood samples were drawn from the femoral artery after intravenous injection of heparin. The animals were then sacrificed, and liver samples were collected. Plasma was separated from whole blood by centrifugation at 1300 ***g*** for 15 min. Plasma TC, TG and LDL-C concentrations were measured using commercial kits and the Hitachi auto-analyser. Hepatic TG was extracted with ethyl acetate and measured with enzymatic methods. LPL, hepatic and plasma FFA concentrations were measured by spectrophotometry using enzymatic methods.

VLDL-TG Secretion Rate Following a 6-h fast, an initial blood sample (0.25 ml) was taken, under anaesthesia with ether, from the orbital venous plexus of both rats and guinea pigs. Rats were injected through the caudal vein and guinea pigs through the jugular vein with 1 ml/kg Triton WR-1339 (200 mg/ml saline), a detergent that prevents intravascular TG catabolism. Blood samples (0.25 ml) were then taken 30, 60 and 120 min after the injection. Plasma was isolated and stored at −20 °C for later TG quantification. The rate of VLDL-TG secretion into the blood was determined from a regression analysis of TG accumulation in plasma *vs.* time. The secretion rate was calculated by multiplying the slope of the regression line by the plasma volume as estimated from body weight (3.5% of the body weight) and was expressed as micromoles per minute.

Assay of Fatty Acid Synthase (FAS) Activity Livers were homogenised in 0.1 M potassium phosphate buffers (pH 7.3) containing 0.07 M $KHCO_3$, 1 M ethylene diamine tetraacetic acid (EDTA), and 1 M DTT. The homogenates were centrifuged at 6800 ***g*** for 30 min and 29700 ***g*** for 60 min, and the supernatants were collected. FAS activity was determined with an Amersham Pharmacia Ultrospec 4300 pro UV–vis spectrophotometer at 37 °C by following the decrease of absorbance at 340 nm due to the consumption of NADPH. The assay mixture contained 100 mM KH_2PO_4–K_2HPO_4 buffer (pH 7.0), 1 mM EDTA, 1 mM DTT, 0.2 mM acetyl-CoA, 0.4 mM malonyl-CoA, and 1.3 mM NADPH in a total volume of 2.0 ml.[11)]

Assay of Acyl CoA: Diacylglycerol Acyltransferase (DGAT) Activity Livers were homogenised in a buffer containing 50 mM Tris–HCl (pH 7.4), 5 mM EDTA and 250 mM sucrose. The homogenates were centrifuged three times at 4 °C to prepare the microsomes. The crude protein concentration in the microsomal fraction was determined using the bicinchoninic acid (BCA) assay with a bovine serum albumin (BSA) standard curve. DGAT assays[12)] were conducted with microsomal protein (20 μg) in the presence of 300 mM sucrose, 10 mM Tris–HCl (pH 7.4), 1 mM EDTA, 10 mM $MgCl_2$, 1 mg/ml BSA (FA-free), 3.33 mM 1,2-dipalmitoyl-*sn*-glycerol and 100 μM palmitoyl-CoA in a total volume of 300 μl. After 10 min at 37 °C, lipids were extracted with chloroform : methanol (2 : 1 vol : vol). The quantification of TG as the result of enzyme reactions was performed by HPLC coupled to an evaporative light-scattering detector.[13)] A Waters 600 Controller was used. Five microlitres of standard TG and the final products were dissolved in chloroform : methanol (2 : 1 vol : vol) and injected. Detection was accomplished with a Waters 2424 ELS Detector. The detector settings (an evaporation temperature of 50 °C and a nitrogen pressure of 30.0 psi) were kept constant in all experiments. The separation of the final products was carried out on a Lichrosphere diol column (250×4.6 mm, 5 μm particle size, PerfectChrom100). The column temperature was maintained at a constant 30 °C throughout the experiments. Details of the mobile phase composition and gradient elution profile are given in Table 2. The flow rate was 0.8 ml/min. The peak area of TG in the HPLC chromatogram was used to quantify the result of the enzyme's reaction. The DGAT activity was then calculated from the amount of the final product (TG).

Assay of Carnitine Palmitoyltransferase-1 (CPT-1) Activity The isolation of mitochondria was performed at 0—2 °C. Livers were homogenised in a 10 mM Tris/HCl medium (pH 7.4) containing 0.25 M sucrose and 1 mM EDTA. Mitochondria were then isolated by differential centrifugation (700 ***g*** for 10 min and 10000 ***g*** for 10 min). The mitochondrial pellets were resuspended in 0.3 M sucrose/10 mM Tris–HCl (pH 7.4)/1 mM EDTA. The protein content of these stock mitochondrial suspensions was determined using the Coomassie brilliant blue staining method. CPT-1 activity assays were carried out at 30 °C in 1 ml of final volume containing 220 mM sucrose, 40 mM KC1, 10 mM Tris–HCl, 1 mM EDTA, 25 μM palmitoyl-CoA and 100 μM carnitine at pH 7.0. The reactions were initiated by the addition of the enzyme. The release of CoA-SH from palmitoyl-CoA was spectrophotometrically assayed using the general thiol reagent DTNB, and the release was equated to the carnitine palmitoyltransferase-1 activity.[14,15)]

RT-PCR Analysis Total RNA was extracted from liver samples using the RNApure high-purity total RNA rapid extraction kit (spin-column, BioTeke). Two micrograms of

RNA was reverse-transcribed with Oligo(dT)18 primer using M-MuLV reverse transcriptase. The final product was used as a PCR template. All PCR primers were designed using the Primer Premier 5.0 and Oligo 6 Demo Software (Table 3). The guinea pig MTTP primers were designed by comparison with the published gene nucleotide sequences of other species (with the following sequence accession numbers: NM001107727, NM000253, L47970, AY217034, U14995). A 2×SYBR real-time PCR premixture (BioTeke) was used to quantify the genes. The PCR reaction was 25 μl, containing 12.5 μl 2×premix, 1 μl sense primer (200 nM), 1 μl antisense primer (200 nM), 1 μl cDNA and 9.5 μl double distilled water. The PCR conditions were 40 cycles of 95 °C for 1 min, 95 °C for 15 s, 56 °C for 40 s, and 76 °C for 10 s. The PCR amplification was performed in a BioRad iQ5. Signals were acquired at the end of amplification in each cycle. The melt-curve analysis was performed using PCR products subjected to a temperature increase from 76 to 95 °C at intervals of 0.1 °C/s. One of the samples was serially diluted 10-fold to prepare the standard templates. The melt-curve analysis was performed using the iQTM5 software. Samples were amplified in triplicate; averages were calculated and the differences in the C_t data of the target genes were evaluated by REST© 2009 software.[16]

Table 2. Ternary Gradient Mobile Phase Composition (%) of HPLC

Time (min)	A	B	C
0	99.2	0.8	0.0
3	97.0	3.0	0.0
5	95.0	5.0	0.0
10	0.0	10.0	90.0
15	0.0	10.0	90.0
16	99.2	0.8	0.0
30	99.2	0.8	0.0

The separation of the final products was carried out on a Lichrosphere diol column. Detection was accomplished with a Waters 2424 ELS Detector to quantify TG as the result of enzyme reactions to determine acyl CoA: diacylglycerol acyltransferase activities. A: hexane; B: 2-propanol; C: methanol.

Table 3. Specific Primers for Genes "Peroxisome Proliferator-Activated Receptor-α (PPARα), Microsomal Triglyceride Transfer Protein (MTTP) and β-Actin" in the Polymerase Chain Reaction

Animals	Gene names	Primer sequence (5′—3′)
Guinea pigs	PPARα	S: CCAGAAAAAGAACCGCAACAAG
		A: AGTTTTGCTTTCTCAGACC
	MTTP	S: AAGCAGAAATTAGAGCTGAAGAC
		A: CAGTGGCTCTGGAAGACCT
	β-Actin	S: AAGGACCTCTATGCCAACAC
		A: TGGAAGGTGGAGAGTGAG
Rats	PPARα	S: TCATACTCGCAGGAAAGACT
		A: ACCTCTGCCTCCTTGTTTTC
	MTTP	S: TACCAGGCTCAGCAAGAC
		A: AGGAAGTTCAAGGCAAAAG
	β-Actin	S: CGTTGACATCCGTAAAGACC
		A: AGCCACCAATCCACACAGAG

Statistical Analysis Statistical significance was assessed using the independent samples *t*-test in SPSS 10.0 for Windows. Differences were considered significant at $p<0.05$.

RESULTS

Plasma Lipid Concentrations in Guinea Pigs and Rats Plasma TC, TG, LDL-C and FFA values were significantly higher (4.2, 1.4, 3.7, 1.5 times, respectively) in the guinea pigs fed the high-fat diet for 4 weeks than in the control group. But rats in the model group, who were fed the same high-fat diet as the guinea pigs, exhibited no changes in plasma TC, TG, LDL-C or FFA concentrations compared to the controls (Table 4).

Hepatic Lipid Concentrations in Guinea Pigs and Rats Both species had higher hepatic TC and FFA concentrations compared with the controls when fed high-fat diets. Although there were no differences in hepatic TG values between the control and model guinea pigs, the rats of the model group had higher hepatic TG concentrations than the controls (Table 5).

Effect of a High-Fat Diet on Plasma LPL and Hepatic CPT-1 Activities in the Two Species Plasma LPL activity was much higher in the groups of both species that were fed high-fat diets than in the control animals ($p<0.01$), and no significant difference between the two species was observed. Hepatic CPT-1 activity increased in the guinea pigs of the model group and decreased in the rats of model group (Table 6).

Table 4. Plasma Total Cholesterol (TC), Triglyceride (TG), Low-Density Lipoprotein Cholesterol (LDL-C) and Free Fatty Acids (FFA) Concentrations in Guinea Pigs and Rats Fed Different Diets

Animals	Groups	Numbers	TC (mmol/l)	TG (mmol/l)	LDL-C (mmol/l)	FFA (μmol/l)
Guinea pigs	Control	9	0.70±0.12	0.60±0.07	0.71±0.09	2447±243
	Model	10	2.96±0.26***	0.84±0.05*	2.60±0.47**	3694±255**
Rats	Control	8	1.69±0.08	0.35±0.03	0.30±0.02	1514±199
	Model	8	1.44±0.05	0.34±0.03	0.23±0.04	1669±319

Values represent mean±S.E. *$p<0.05$; **$p<0.01$; ***$p<0.001$, compared to controls.

Table 5. Hepatic Total Cholesterol (TC), Triglyceride (TG) and Free Fatty Acids (FFA) Concentrations in Guinea Pigs and Rats Fed Different Diets

Animals	Groups	Numbers	TC (mg/g)	TG (mg/g)	FFA (μmol/g prot)
Guinea pigs	Control	9	1.16±0.04	19.56±2.98	3218±819
	Model	10	1.74±0.11***	25.26±4.05	3958±561*
Rats	Control	8	1.44±0.08	14.27±1.00	1988±362
	Model	8	1.79±0.08**	28.36±3.08***	2457±350*

Values represent mean±S.E. *$p<0.05$; **$p<0.01$; ***$p<0.001$, compared to controls.

VLDL-TG Secretion Rate The accumulation of plasma TG after the administration of Triton occurred more quickly in the guinea pigs in the model group than in those in the control group, but it was significantly slower in rats in the model group compared to those in the control group (Fig. 1). Thus, the *in vivo* VLDL-TG secretion rate from the liver was significantly higher in the guinea pigs in the model group than in those in the control group ($p<0.05$) and much lower in the rats in the model group than in those in the control ($p<0.05$) (Fig. 2).

Hepatic FAS and DGAT Activities in Guinea Pigs and Rats Fed High-Fat Diets There was a consistent change in hepatic FAS activity across the animal species. The activity was 26% lower than the controls in guinea pigs fed high-fat diets and 51% lower than the controls in rats fed high-fat diets. As shown in an HPLC chromatogram (Fig. 3) obtained by the evaporative light-scattering detector, the peak area of the final product (TG) was larger in the guinea pigs in the model group than in those in the control group. The opposite was true for the rats. Thus, DGAT activity, as calculated by the amount of TG, increased in guinea pigs fed high-fat diets and decreased in rats fed high-fat diets (Table 7).

Hepatic MTTP and PPARα: mRNA Expression in Guinea Pigs and Rats In the conventional melt-curve analysis, all the PCR products for each gene generated a single peak, suggesting that there was a single amplified product of the target gene. The relative quantitative results showed that hepatic MTTP and PPARα mRNA expression was significantly higher in the guinea pigs fed high-fat diets than in the controls ($p<0.05$). A slightly decreased expression of hepatic PPARα compared to the controls was found in the rats fed high-fat diets. There was no difference in hepatic MTTP

Table 6. Plasma Lipoprotein Lipase (LPL) and Hepatic Carnitine Palmitoyltransferase-1 (CPT-1) Activities in Guinea Pigs and Rats Fed Different Diets

Animals	Groups	Numbers	LPL (U/L)	CPT-1 (pmol·min^{-1} /mg prot)
Guinea pigs	Control	9	8.95±0.52	64.28±1.65
	Model	10	12.50±0.37***	70.30±1.68*
Rats	Control	8	6.89±0.67	82.41±3.52
	Model	8	10.37±0.69**	69.43±1.27*

Values represent mean±S.E. *$p<0.05$; **$p<0.01$; ***$p<0.001$, compared to controls.

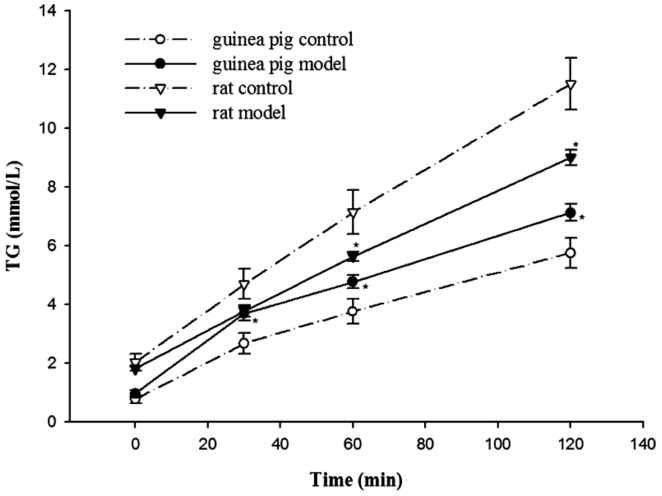

Fig. 1. General Trend in the Accumulation of Plasma Triglycerides after Administration of Triton in Guinea Pigs and Rats

The accumulation of plasma TG after the administration of Triton occurred more quickly in the guinea pigs in the model group than in those in the control group, but it was significantly slower in rats in the model group compared to those in the control group. Values are the mean±S.E. of ten guinea pigs or eight rats each group, significantly different from control: *$p<0.05$.

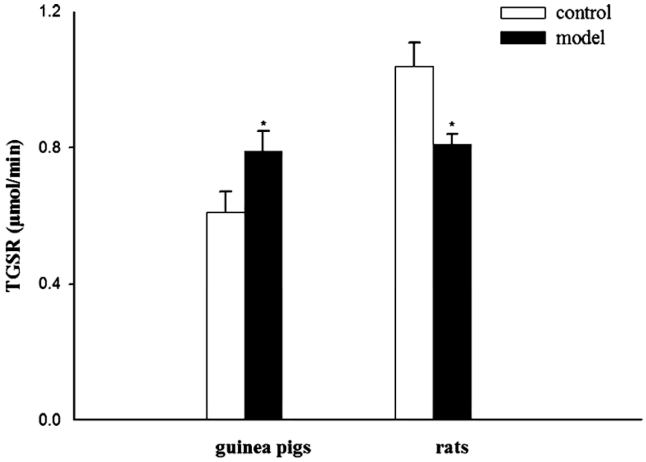

Fig. 2. Hepatic Very-Low-Density Lipoprotein-Triglyceride (VLDL-TG) Secretion Rate (TGSR) in Guinea Pigs and Rats Fed Different Diets

Values are the mean±S.E. of ten guinea pigs or eight rats each group. VLDL-TG secretion rate from the liver was significantly higher in the guinea pigs in the model group than in those in the control group (*$p<0.05$) and much lower in the rats in the model group than in those in the control (*$p<0.05$).

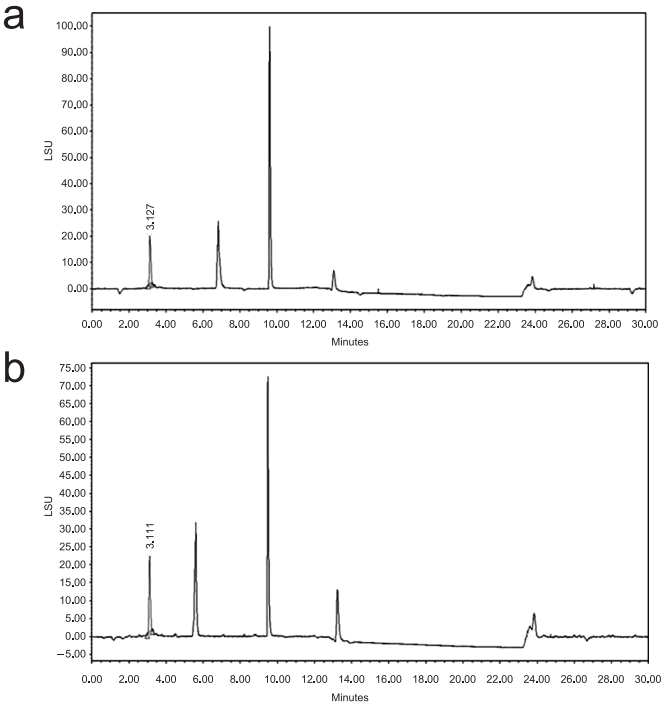

Fig. 3. HPLC Chromatogram Obtained by the Evaporative Light-Scattering Detector, to Quantify the Final Product (TG) in Enzyme Reactions Catalyzed by Microsomes in Guinea Pigs

The final products were dissolved in chloroform–methanol (2 : 1, vol/vol) and injected. Detection was accomplished as described in the text. The peak area of TG was larger in the guinea pigs in the model group (b, retention time: 3.111 min, area: 108184) than in those in the control group (a, retention time: 3.127 min, area: 86554).

Table 7. Hepatic Fatty Acid Synthase (FAS) and Acyl CoA: Diacylglycerol Acyltransferase (DGAT) Activities in Guinea Pigs and Rats Fed Different Diets

Animals	Groups	Numbers	FAS (nmol·min^{-1}/mg prot)	DGAT (nmol·min^{-1}/μg prot)
Guinea pigs	Control	9	1.94±0.11	0.019±0.005
	Model	10	1.42±0.11**	0.029±0.008**
Rats	Control	8	6.06±0.24	0.150±0.037
	Model	8	2.99±0.11***	0.112±0.017*

Values represent mean±S.E. $*p<0.05$; $**p<0.01$; $***p<0.001$, compared to controls.

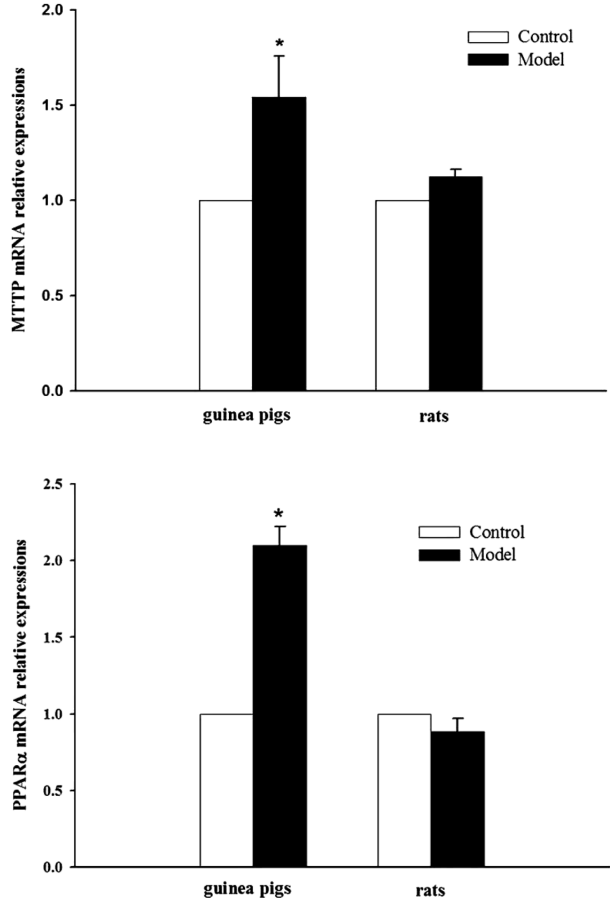

Fig. 4. Hepatic Microsomal Triglyceride Transfer Protein (MTTP) mRNA Expression and Peroxisome Proliferator-Activated Receptor-α (PPARα) mRNA Expressions in Guinea Pigs and Rats Fed Different Diets

Results are the mean±S.E. from eight guinea pigs or rats per group. All real-time quantitative PCR reactions were carried out in triplicate. Relative gene expressions of MTTP and PPARα were expreessed as the ratio of MTTP or PPARα gene expression to β-actin gene expression. Hepatic MTTP and PPARα mRNA expression was significantly higher in the guinea pigs fed high-fat diets than in the controls ($*p<0.05$).

expression between rats of the model and control groups. (Fig. 4).

DISCUSSION

Guinea pigs and rats are both commonly used animal models for hyperlipidemia studies. However, the results presented here show that the response of guinea pigs to a high-fat diet is distinct from that of rats, suggesting a difference in fat metabolism between the two. Treatment with a high-fat diet (0.1% cholesterol and 10% lard) for 4 weeks led to significant increases in plasma TC, LDL-C, TG and FFA in guinea pigs but not in rats. Although hepatic TC and FFA concentrations in both species were enhanced by high-fat diets, hepatic TG concentration was not increased by a high-fat diet in guinea pigs, whereas it was in rats.

The plasma and hepatic TG levels depend on hepatic TG synthesis, VLDL-TG secretion and lipolysis. There are two major pathways for triglyceride biosynthesis: the glycerol phosphate pathway and the monoacylglycerol pathway. The linking of diacylglycerol and fatty acyl CoA, which is catalyzed by DGAT, is the common final step for the two pathways and is the rate-limiting step for TG biosynthesis.[17,18] In this work, we found that hepatic DGAT activity increased in response to a high-fat diet in guinea pigs but decreased in rats, suggesting that the hepatic TG biosynthesis response to a high-fat diet is quite different in guinea pigs and rats. It has been reported that hepatic TG biosynthesis is of great importance in the secretion of VLDL.[19] The increased hepatic TG biosynthesis in guinea pigs fed a high-fat diet might therefore lead to an increase in VLDL secretion, a hypothesis supported by the VLDL-TG secretion rate assay. Moreover, the level of hepatic MTTP mRNA, which is responsible for the transport of neutral lipid (TG and cholesterol ester) between the phospholipid surfaces of the endoplasmic reticulum[20] and which plays a vital role in the formation and secretion of VLDL,[19,21] was upregulated by a high-fat diet in guinea pigs but not in rats. Therefore, the upregulated hepatic DGAT activity and MTTP transcription in response to a high-fat diet in guinea pigs could account for their higher VLDL secretion rate compared with rats.

Plasma FFA is an essential component of plasma lipids and is released during the lipolysis of adipose tissue and TG-rich lipoproteins.[22] Higher plasma TG concentrations are directly associated with higher plasma FFA concentrations. This association might explain why the plasma FFA concentrations in guinea pigs fed a high-fat diet were much higher than those in rats. Furthermore, excess FFA might be a major factor in increased hepatic VLDL-TG secretion.[23,24] Thus, excess plasma FFA could stimulate hepatic VLDL production and secretion. This hypothesis is consistent with our experimental results. An increased VLDL-TG secretion rate was associated with a higher plasma FFA concentration in guinea pigs fed a high-fat diet, whereas a decreased VLDL-TG secretion rate was associated with unchanged plasma FFA in rats. Since hepatic FAS activity was decreased compared to controls in both guinea pigs and rats fed a high-fat diet, the increase in FFA was mainly derived from exogenous FFA feeding.

Hepatic TG content is also closely related to the rate of hepatic mitochondrial fatty acid β-oxidation. Long-chain CPT-1, which catalyzes the transfer of acyl units from palmitoyl-CoA (and other long-chain acyl-CoA esters) to carnitine, is the first rate-limiting enzyme in mitochondrial fatty acid oxidation.[25] Wang *et al.* reported that the activity and mRNA levels of hepatic CPT were downregulated in rats fed a 10% corn oil diet with 1% cholesterol for 4 weeks.[26] In our study, 4 weeks of a high-fat diet significantly decreased hepatic CPT-1 activity in rats, a result which is in accord with the previous report. However, the hepatic CPT-1 activity in guinea pigs was increased by a high-fat diet. Moreover, the hepatic mRNA level of PPARα, an important factor in fatty

acid oxidation,[27,28] was significantly increased in guinea pigs and slightly decreased in rats after 4 weeks of a high-fat diet. Based on the different responses of CPT-1 and PPARα to a high-fat diet in the two species, the effects of a high-fat diet on the rate of mitochondrial fatty acid β-oxidation in guinea pigs and rats might be different. Since hepatic lipid deposition is closely related to the rate of hepatic mitochondrial fatty acid β-oxidation, this observation may partially explain why hepatic TG concentration was unchanged in guinea pigs and increased in rats fed a high-fat diet.

LPL is an enzyme that plays a vital role in plasma triglyceride regulation. It promotes plasma TG clearance by hydrolyzing triglycerides in chylomicrons and VLDL.[29] Previous studies have shown that the development of hypertriglyceridemia is closely associated with the downregulation of LPL activity.[30] In our research, however, the activity of plasma LPL was upregulated by a high-fat diet in both guinea pigs and rats, a result which could be induced by increasing exogenous TG synthesis in response to dietary fat. In any case, this result suggests that the hypertriglyceridemia in guinea pigs fed a high-fat diet may not result from downregulation of LPL activity.

In summary, the data presented here suggest that there are some differences between guinea pigs and rats in the response of TG metabolism to high-fat diets (0.1% cholesterol and 10% lard). These differences are found in TG biosynthesis, VLDL secretion and lipolysis, which hadn't been reported. First, the increased biosynthesis of TG and excess plasma FFA stimulate VLDL-TG secretion in guinea pigs. The upregulation of hepatic MTTP transcription in guinea pigs may be another factor stimulating VLDL secretion. The increased VLDL-TG secretion, in turn, leads to an elevation of plasma TG concentration. Second, the rate of hepatic TG synthesis was in equilibrium with the rate of VLDL-TG secretion in guinea pigs, whereas it exceeded the rate of VLDL secretion in rats, which resulted in increased hepatic lipid deposition in rats but not in guinea pigs. Third, a high-fat diet increases mitochondrial fatty acid β-oxidation in guinea pigs by enhancing hepatic CPT-1 activity and PPARα transcription but decreases mitochondrial fatty acid β-oxidation in rats by suppressing hepatic CPT-1 activity and PPARα transcription. This difference leads to different hepatic TG concentrations in guinea pigs compared to rats. The different response of TG metabolism to a high-fat diet in guinea pigs and rats suggests that guinea pigs could be a better hypertriglyceridemia animal model than rats for research on lipid metabolism disorders and hypolipidemic drugs.

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REFERENCES

1) Fernandez M. L., Volek J. S., *Nutr. Metab.* (London), **3**, 17 (2006).
2) Liu X. M., Wu F. H., *Journal of Chinese Integrative Medicine*, **2**, 132—134 (2004).
3) Fernandez M. L., McNamara D. J., *J. Nutr.*, **121**, 934—943 (1992).
4) Torres-Gonzalez M., Leite J. O., Volek J. S., Contois J. H., Fernandez M. L., *J. Nutr. Biochem.*, **19**, 856—863 (2008).
5) Fernandez M. L., *J. Nutr.*, **131**, 10—20 (2001).
6) Lin M. H., Lu S. C., Huang P. C., Liu Y. C., Liu S. Y., *Ann. Nutr. Metab.*, **49**, 386—391 (2005).
7) Spady D. K., Cuthbert J. A., *J. Biol. Chem.*, **267**, 5584—5591 (1992).
8) Li D. W., Zhang L., Xia Z. L., *Chinese Journal of Clinical Rehabilitation*, **10**, 145—147 (2006).
9) Harris W. S., *Am. J. Clin. Nutr.*, **65** (Suppl.), 1611S—1616S (1997).
10) Li J. L., Gao N. N., Yang R. M., *Acta Lab. Anim. Sci. Sin.*, **17**, 115—119 (2009).
11) Tian W. X., Jiang R. F., Wu H. B., Shi Y. H., Wang Y. H., *Chin. Biochem. J.*, **10**, 413—419 (1994).
12) Siloto R. M., Truksa M., He X., McKeon T., Weselake R. J., *Lipids*, **44**, 963—973 (2009).
13) Perona J. S., Ruiz-Gutierrez V., *J. Sep. Sci.*, **27**, 653—659 (2004).
14) Markwell M. A., McGroarty E. J., Bieber L. L., Tolbert N. E., *J. Biol. Chem.*, **248**, 3433—3440 (1973).
15) Bremer J., Woldegiorgis G., Schalinske K., Shrago E., *Biochim. Biophys. Acta*, **833**, 9—16 (1985).
16) Pfaffl M. W., Horgan G. W., Dempfle L., *Nucleic Acids Res.*, **30**, e36 (2002).
17) Villanueva C. J., Monetti M., Shih M., Zhou P., Watkins S. M., Bhanot S., Farese R. V. Jr., *Hepatology*, **50**, 434—442 (2009).
18) Mayorek N., Grinstein I., Bar-Tana J., *Eur. J. Biochem.*, **182**, 395—400 (1989).
19) Gibbons G. F., Wiggins D., Brown A. M., Hebbachi A. M., *Biochem. Soc. Trans.*, **32**, 59—64 (2004).
20) Gordon D. A., Jamil H., Sharp D., Mullaney D., Yao Z., Gregg R. E., Wetterau J., *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 7628—7632 (1994).
21) Gregg R. E., Wetterau J. R., *Curr. Opin. Lipidol.*, **5**, 81—86 (1994).
22) Chung B. H., Tallis G. A., Cho B. H., Segrest J. P., Henkin Y., *J. Lipid Res.*, **36**, 1956—1970 (1995).
23) Julius U., *Exp. Clin. Endocrinol. Diabetes*, **111**, 246—250 (2003).
24) Coppack S. W., Jensen M. D., Miles J. M., *J. Lipid Res.*, **35**, 177—193 (1994).
25) Brosnan J. T., Kopec B., Fritz I. B., *J. Biol. Chem.*, **248**, 4075—4082 (1973).
26) Wang Y. M., Wang J. F., Xue C. H., *Ying Yang Xue Bao*, **29**, 530—534 (2007).
27) Pineda Torra I., Gervois P., Staels B., *Curr. Opin. Lipidol.*, **10**, 151—159 (1999).
28) Wahli W., Braissant O., Desvergne B., *Chem. Biol.*, **2**, 261—266 (1995).
29) Goldberg I. J., *J. Lipid Res.*, **37**, 693—707 (1996).
30) Benhizia F., Hainault I., Serougne C., Lagrange D., Hajduch E., Guichard C., Malewiak M. I., Quignard-Boulangé A., Lavau M., Griglio S., *Am. J. Physiol.*, **267**, E975—E982 (1994).