

Full Length Research Paper

Characterization and mechanisms of lipid metabolism in high-fat diet induced hyperlipidemia in Mongolian gerbil (*Meriones unguiculatus*)

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Rodent are common animal models for hyperlipidemia. In the present study, the differences in lipid metabolism between gerbils and rats were investigated. Feeding a high-fat diet led to a significant increase in serum total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDLC) and high-density lipoprotein cholesterol (HDLC) in gerbils, and were found in a time-dependent manner during 0 to 16 weeks' feeding. Hepatic lipid vacuolization and even fibrosis in gerbils were greatly formed in response to the high fat diet with the characteristic of serum LDLC increase, while those remained lower changed or unchanged in rats. Furthermore, serum lecithin cholesterol acyl transferase (LCAT) activities in the hyperlipidemia gerbils were significantly higher than those in the normal ones, which were also in line with increased LDLC-TG secretion rate and impaired hepatic function in gerbils in response to the high-fat diet. Therefore, gerbils were considered to be more sensitive to high fat diet, less time-consuming in forming hyperlipidemia. Similar response in increased LDLC levels to cholesterol as human and may warrant further application as a possible model for drug evaluation and lipid metabolism.

Key words: Mongolian gerbil, hypercholesterolemia, low-density lipoprotein cholesterol, lecithin cholesterol acyl transferase.

INTRODUCTION

Hypercholesterolemia and hypertriglyceridemia, disorders in the lipoprotein metabolism, are characterized by increased levels of serum low-density lipoprotein cholesterol (LDL-C). Several reports have indicated that they are the dominant risk factors for the development of cardiovascular disease, such as atherosclerosis and coronary heart disease (Pollak, 1987; Kawasaki et al., 1997).

Since approximately two-third of serum total cholesterol is normally transported as cholesterol esters in LDL fraction, there is a strong correlation between serum total cholesterol levels and serum LDLC levels. Despite advances in the use of hypolipidemic drugs (such as fibrates and statins), some potential adverse effects and great drug dependence are still a major problem in treated patients (Alsheikh-Ali et al., 2004). Therefore, novel drug therapies to address dyslipidemia are still being sought, and a well characterized and quantitative animal model is an essential tool in their development (Suckling and Jackson, 1993; Kourounakis et al., 2002; Mobasher and Cassidy, 2010).

Several preclinical models have been used to explore hypolipidemic effects of novel compounds on hyperlipidemia, most frequently the male rat or mice model (for avoi-

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Abbreviations: TC, total cholesterol; TG, triglycerides; LDLC, low density lipoprotein cholesterol; HDLC, high density lipoprotein cholesterol; GLC, glucose.

ding influence of estrogen on lipid metabolites). Although rats have been extensively used in biomedical research to generate data on metabolic diseases, they have limitations as models of lipid metabolism in humans. Lipid metabolism particularly in changes of lipoproteins and serum cholesterol levels in response to dietary factors differ significantly between rats and humans (Ramachandran et al., 2003; Babin and Gibbons, 2009). Thus, using rat as an experimental model in studying hyperlipidemia hinders extrapolation of data since fatty acids and cholesterol play important roles in the etiology of diseases.

Mongolian gerbil (*Meriones unguiculatus*) is the most widely used species of gerbil in the laboratory (Li et al., 2010; Zibael et al., 2010; Kovalenko et al., 2011). Although, there are several other species in the genera *Gerbillus* (gerbils, 54 to 62 species) and *Meriones* (sand rats, 14 species), the species used in experiment is limited to *M. unguiculatus*. Earlier investigations have demonstrated that the gerbil responds promptly to dietary fat and cholesterol typical of human intakes with a marked rise in serum cholesterol and hepatic cholesterol esters. In contrast, feeding cholesterol to rat only cause a relatively small rise in liver cholesterol-ester content, while serum cholesterol hardly shows any increase at all (Temmerman et al., 1989; Suckling and Jackson, 1993). Thus, the cholesterol metabolism of the gerbil on different high-fat diet resembles the human response. However, the mechanisms involved in high fat diet-induced hyperlipidemia in the gerbil have not been sufficiently elucidated. To further understand the pathogenesis of hypercholesterolemia and provide a more appropriate animal model of hyperlipidemia for drug evaluation, this study was to characterize lipid metabolism and hepatocyte morphology after high fat feeding, and to investigate the changes of lecithin cholesterol acyl transferase (LCAT) in this particular model of progressive adaptation and degeneration.

MATERIALS AND METHODS

Animals and diet

Male gerbils (*M. unguiculatus*) weighing 50 to 60 g, and Sprague-Dawley rats weighing 160 to 200 g were obtained from Key Laboratory of Experimental Animal, Zhejiang Academy of Medical Sciences, China. All animals were housed four per standard cage, on 12 h light/dark cycle; and air temperature was maintained at $22 \pm 2^\circ\text{C}$. Experiments were carried out in accordance with local guidelines and approved by the ethics committee for research on laboratory animal use of the institution [No. SCXK (Zhe) 2008-0116].

All animals (rats and gerbils) were fed with a commercially available standard diet for one week in the experimental environment before the experiments were conducted. Once they had adapted to the environment, the animals in the normal control group were fed with basic diet, while the others were fed with high-fat diet (~1.67 Kcal/g), which was composed with 80.5% basic diet, 2% cholesterol, 7% lard, 10% yolk powder and 0.5% bile salts as previously reported (Feng et al., 2011).

Experimental design

At the beginning, 4th, 8th, 12th and 16th weeks of the experimental

period, eight gerbils or eight rats each group were randomly taken 2 h after fasting and anaesthetized with chloral hydrate. Subsequently, the animals were sacrificed, and blood and livers were taken for biochemical analysis. Hepatic index was given as follows:

$$\text{Hepatic index} = \frac{\text{liver weight}}{\text{body weight}}$$

It is a classic parameter for hepatocytes intracellular fat accumulation (hepatosteatosis).

Hepatic morphology

Material for the histological examination was taken from the liver of each animal after sacrificed. The obtained tissue specimens were weighted and then put in a buffer solution of 10% neutral-buffered formalin. After fixation, the fixed tissue were processed routinely for paraffin embedding, cut into 4 μm thick sections and stained with hematoxylin and eosin, and Sirius Red-chrysolepic acid. The stained areas were viewed using an optical microscope at $\times 100$.

Biochemical analysis

Blood samples were collected and centrifuged at $3500 \times g$ for 15 min to obtain serum. The levels of serum total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDLC), high density lipoprotein cholesterol (HDLC) and glucose (GLC) were determined by an automatic chemical analyzer (HITACHI 7100, Japan) with commercially available enzymatic kits (Huifeng Science and technology Co. LTD., Shanghai, China) according to the manufacturer's instructions. The levels of serum and hepatic LCAT were determined by using commercially available ELISA kits (Tangpu biotech Co. LTD., Hangzhou, China).

Statistical analysis

All parameters were recorded for individuals within all groups. All data were shown as mean \pm standard deviation (SD). Statistical comparisons of data were carried out using the ANOVA and t-test. A two-tailed $P < 0.05$ was considered to be significant. Correlations between concentrations of serum lipids (TG, TC, LDLC and HDLC) and LCAT were calculated using the Pearson coefficient analysis. All statistical analysis was performed using the SPSS 17.0 system.

RESULTS

High fat diet-induced hyperlipidemia on viscera and blood lipid profiles

As shown in Figure 1, over a period of four weeks of feeding, the levels of serum TC, TG, LDLC, HDLC and glucose in high fat diet-fed gerbils were significantly higher than those in the 0W group, which indicated that the model was successful in inducing hyperlipidemia in gerbils. Compared with the lipids profiles of hyperlipidemia gerbils, those biomarkers in the rats were not significantly increased in response to the high fat diet. Serum levels of TC, LDLC, HDLC and glucose in rats after the feeding of four weeks were not significantly increased

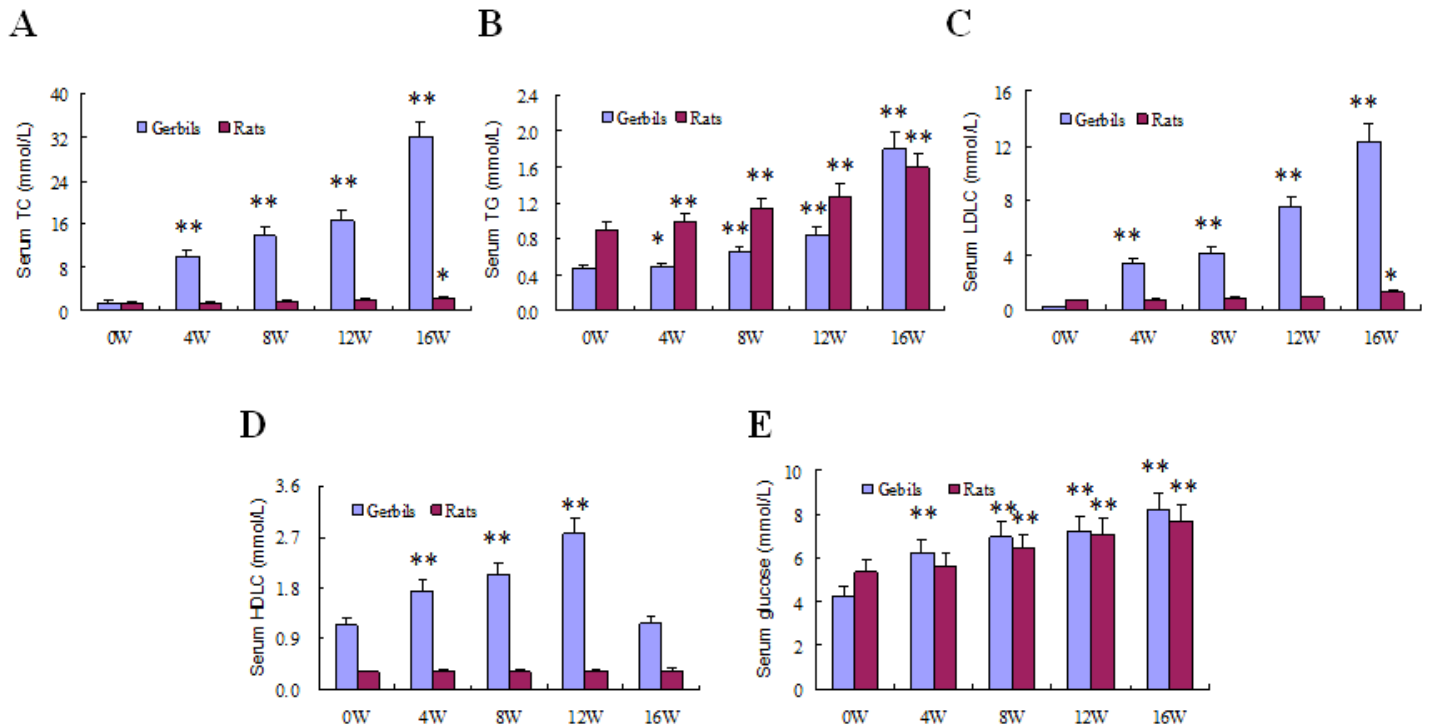


Figure 1. Graphs illustrating the influence on the serum levels of TC (A), TG (B), LDLC (C), HDLC (D), and ALT (E) in hyperlipidemia gerbils and hyperlipidemia rats induced by high-fat diet. Values are the means \pm SD ($n = 8$). 0 W, Compared with the normal animals; * $P < 0.05$; ** $P < 0.01$.

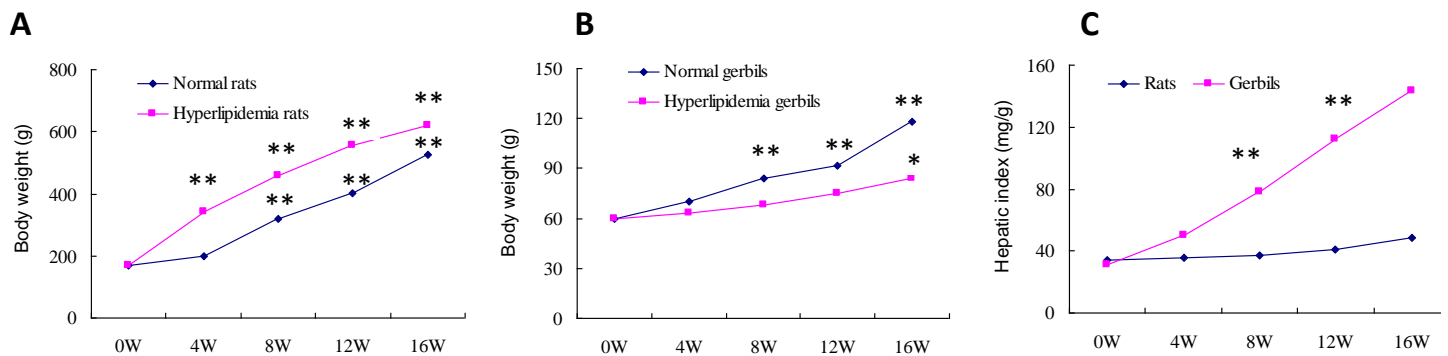


Figure 2. Body weights (A, rats; B, gerbils) and hepatic indexes (C) of high-fat diet fed animals at different periods. Values were the means \pm SD ($n = 8$). 0W, Compared with the normal animals. * $P < 0.05$; ** $P < 0.01$.

compared with those in the 0W group ($P > 0.05$). It indicated that the gerbils seem to be more sensitive to high fat diet than rats. As the disease progresses, the responses increased as the weeks of feeding increased; however, the levels of serum HDLC reached a maximum at 12 weeks of treatment, after which time began to decrease. The levels of serum HDLC after 16 weeks feeding were even significantly lower than those in other periods (at 4, 8 and 12 weeks), and no significant differences of serum HDLC concentrations were found compared to the 0W group ($P > 0.05$). In addition, during the experimental period, the levels of serum LDLC in

hyperlipidemia gerbils were also constantly higher compared to the 0W group. The rate constant of LDLC $_{0-16 \text{ weeks}}$ [$0.086 \pm 0.009 \text{ U}/(\text{L}\cdot\text{week})$] were also higher ($P < 0.01$) than that of HDLC $_{0-16 \text{ weeks}}$ [$0.057 \pm 0.005 \text{ U}/(\text{L}\cdot\text{week})$].

The changes of hepatic indexes and body weights are presented graphically in Figure 2 corresponding to the serum TC and TG responses. There were no significant changes in the growth rate of body weights between rats (Figure 2A) and gerbils (Figure 2B). However, the growth rate of rat hepatic index was likely to be uniformly gentleness. Unlike the trends of rats fed, an actual res-

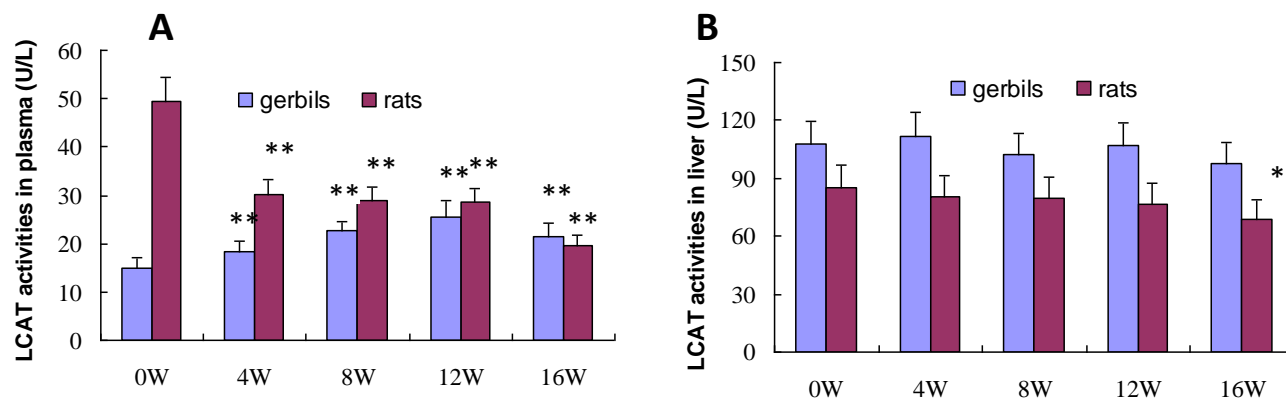


Figure 3. Graphs illustrating the influence on the levels of serum LCAT (A) and liver LCAT (B) in hyperlipidemia gerbils induced by high-fat diet. Values are the means \pm SD (n = 8). 0W, Compared with the normal animals. * P <0.05; ** P <0.01.

Table 1. Pearson correlation coefficients between levels of serum lipids (TG, TC, LDL-C and HDL-C) and LCAT in high-fat diet fed gerbils (n=40).

Serum lipid	LDLC	HDLC	TG	TC	LCAT
LDLC	1	-0.515*	0.138	-0.129	0.583**
HDLC	-	1	-0.192	-0.023	-0.218
TG	-	-	1	0.816**	0.524**
TC	-	-	-	1	0.483**
LCAT	-	-	-	-	1

* Significant at $P < 0.05$, ** Significant at $P < 0.01$.

ponse of gerbil hepatic index in the same condition was shown from the beginning to the 16th week, indicating that gerbil has the most sensibility to high fat diet (Figure 2C).

Property analysis of serum and liver LCAT

The results of the LCAT activities in plasma and liver of hyperlipidemia gerbils are presented in Figure 3. With the development of the hyperlipidemia, the activities of serum LCAT in gerbils during 0 to 16 weeks were significantly increased compared with those in the 0W group (all $P < 0.01$) and the responses were in a time-dependent manner; but the levels of hepatic LCAT at four to 16 weeks had no significant increase (all $P > 0.05$). However, the activities of serum LCAT in rats were significantly decreased in a time-dependent manner. To clarify the different characteristic of gerbil lipid metabolism, the associations of serum LCAT activities with levels of serum lipids, that is, TC, TG, LDLC and HDLC were evaluated as shown in Table 1. A high degree of correlation ($P < 0.01$), well matched with the predicted linear model with the Pearson coefficients of determination (r) of 0.583, was found between serum LCAT and serum LDLC concentrations in the hyperlipidemia gerbils, while between LCAT and TG, it

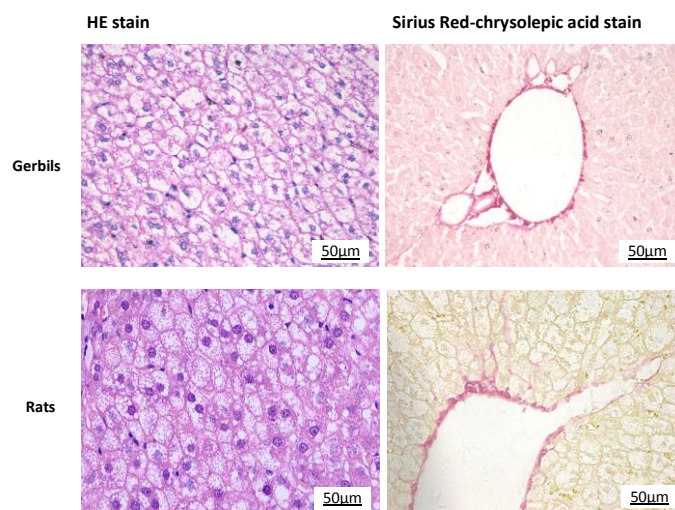


Figure 4. Hepatic histological changes of hyperlipidemia animals ($\times 400$).

was 0.524 ($P < 0.01$), and between LCAT and TC was 0.453 ($P < 0.01$). However, no correlation ($P > 0.05$) was found between serum LCAT activities and serum HDLC concentrations.

Histological examination

Significant morphological changes were observed in the hyperlipidemia gerbils and rats as shown in Figure 4. Liver sections in the hyperlipidemia gerbils and rats both showed less cells and lipid vacuolization. Accumulation of hepatic lipid droplets appeared to be relatively higher in the livers of gerbils than that in the rats as previous studies showed (Woo et al., 2009; Aissaoui et al., 2011). Over a period of 12 weeks, hepatic fibrosis (type I collagen and type III collagen) stained with red in Figure 4 was shown in hepatic tissue of hyperlipidemia gerbils while high fat diet-fed rats had no or little pathological

feature.

DISCUSSION

Mongolian gerbil has been used with increasing popularity as a small animal model in studies of lipid metabolism because of its closer similarities to human being and as a sensitive species to study atherosclerosis (Wasan et al., 2001). Although rats and mice have been extensively used in biomedical research to generate data on cardiovascular diseases, lipid metabolism particularly in relation to high fat diet and changes in serum cholesterol levels in response to dietary factors differ significantly between rats and humans (Suckling and Jackson, 1993). Studies have shown that gerbils change in blood cholesterol concentrations in response to dietary fat type and content are closer to that of humans than rats (Ramachandran et al., 2003). However, earlier investigations also demonstrated that gerbils can tolerate diet containing 199 fat and cholesterol typical of human intakes (Temmerman et al., 1988). To clarify these confusions, a 16-week feeding study with gerbils was conducted to evaluate the influence of high fat diet on serum lipid profiles.

In present study, we characterized serum lipid components (TC, TG, LDLC and HDLC) and activities of LCAT in serum and hepatic tissue in the hyperlipidemia gerbil. After four weeks, significant increases of lipid components in the high fat diet fed gerbil were found. Except an acute decrease of HDLC during 12 to 16 weeks, responses of other serum lipid levels increased positively as feeding time increased. The serum samples turned to be lactescent, indicating that serum TG was found to proportionally increase in a time-dependent manner. Compared with rats fed on the same condition, many large fat droplets were shown in the hepatic tissues of gerbils, indicating the fatty infiltration and degeneration of liver cells caused by fat feeding. After 16 weeks feeding, the structures of hepatic lobules with hepatic fibrosis were destructed and hepatic sinusoids disappeared in hepatic tissues of gerbils. Collagen fibers were formed between portal area and pseudolobule, while rats failed to develop these structural and functional pathological changes in liver. It confirmed the sensitivity of serum cholesterol concentrations to dietary fat content in gerbils.

Furthermore, accumulating evidences have demonstrated that fat and cholesterol can cause liver injury (Nikas et al., 2001; Tessari et al., 2009). In the present study, serum ALT and AST activities, the diagnostic markers of hepatic damage, were elevated by high fat diet treatment. Moreover, the histological changes of liver, such as hepatic fibrosis, had been observed in treated animals. As the disease progresses, the responses increased as feeding time increased during 0 to 12 weeks; however, the levels of serum ALT and AST reached a maximum at 12 weeks of treatment, after which time both began to go down. These findings indicate that long-term intake of high fat

diet could initiate chronic damage of hepatocyte and even cause hepatic fibrosis. It was in accordance with pathological changes observed above. However, these results could not be observed in treated rats.

LCAT, is an interfacial enzyme essential for the esterification of serum TC, secreted by hepatocytes and released to plasma in a relatively loose association with large HDLs. It is generally accepted that higher serum cholesterol esterification rate is not necessarily protective, but the role of LCAT may depend on the concentration and quality of serum HDL and LDL particles (Dobiášová and Frohlich, 1999; Zannis et al., 2006). As shown in Figure 2, with the increased levels of serum TC, the activities of serum LCAT increased more significantly during 0 to 16 weeks compared to the 0W group. It pointed out an obvious raise in serum lipids levels (TG and LDLC concentrations) and a sharp shift in liver biomarkers (ALT and AST activities). It is well-known that the rats transport most of their serum cholesterol in the HDLC fraction, have higher ability of serum cholesterol clearance and could not develop hypertriglyceridemia in response to cholesterol feeding (Yang et al., 2011). Confirmed by correlation analysis in the present study, the activities of serum LCAT positively correlated with hepatic function and TG biosynthesis after long-term feeding. Moreover, it was worth noting that during the experimental period, the rate constant of LDLC_{0-16 weeks} were higher ($P < 0.01$) than HDLC_{0-16 weeks}. Unlike the transportation of serum cholesterol in rats, there was an obvious raise of serum LDLC in gerbils as the same response to dietary factors as human beings. It has been reported that hepatic TG biosynthesis is great important in the secretion of VLDL (Gibbons et al., 2004). The increased hepatic TG biosynthesis in gerbils fed a high-fat diet might therefore lead to an increase in VLDL secretion. Moreover, the levels of LCAT activities are responsible for the transport of TG and cholesterol ester, playing a vital role in the formation and secretion of VLDL. Therefore, the up-regulated LCAT activity in response to a high-fat diet in gerbils could account for their higher VLDL secretion rate compared with rats. These results suggest that lipid metabolism in gerbils was accompanied by shifted LCAT activities and impaired hepatic function, which were in line with an increased LDLC-TG secretion rate in gerbils in response to a high-fat diet, but these probably was due to the compensatory increase in hepatic function, which typically failed to be seen in rats (Yang et al., 2011).

Conclusions

Feeding a high-fat diet for four weeks led to a significant increase in TC, TG, LDLC and HDLC in gerbils. As the feeding time increased, levels of lipids (TC, TG, LDLC and HDLC), ALT and AST were significantly increased in a time-dependent manner. By contrast, hepatic lipid vacuolization and even fibrosis in gerbils were greatly formed with the characteristic of constant increase of serum

LDLC levels, while rats failed in response to the high fat diet. Furthermore, serum LCAT activities in gerbils fed a high-fat diet were significantly higher than those in the normal control group, which was also in line with increased LDLC-TG secretion rate and impaired hepatic tolerance in gerbils in response to the high-fat diet. These findings would establish the suitability of the high fat-fed male gerbil as a novel hyperlipidemia model for characterization of therapies directed at lipid dysfunction.

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