**NOTE** *Internal Medicine*

# Experimental Hyperlipemia Induces Insulin Resistance in Cats

Naohito NISHII[1)]*, Haruka MAEDA[2)], Yusuke MURAHATA[2)], Aya MATSUU[2)] and Yoshiaki HIKASA[2)]

[1)]*Laboratory of Veterinary Pharmacotherapeutics, Faculty of Agriculture, Tottori University, 4–101 Koyama-minami, Tottori 680–5883, Japan*
[2)]*Laboratory of Veterinary Internal Medicine, Faculty of Agriculture, Tottori University, 4–101 Koyama-minami, Tottori 680–5883, Japan*

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ABSTRACT. The effect of experimental hyperlipemia on insulin sensitivity was evaluated in seven healthy cats. Serum triglyceride and free fatty acid concentrations were significantly ($P$<0.05) higher when lipid-heparin was administered (2,894 ± 1,526 mg/d*l* and 4.54 ± 0.70 mEq/*l*, respectively) than when saline was administered (70 ± 42 mg/d*l* and 0.22 ± 0.08 mEq/*l*, respectively). A glucose clamp test revealed that the mean glucose infusion rate when lipid-heparin was administered (5.80 ± 0.67 mg/kg/min) was significantly ($P$<0.05) lower than when saline was administered (8.52 ± 1.83 mg/kg/min). These results suggest that experimental hyperlipemia induced insulin resistance in the healthy cats.

KEY WORDS: feline, hyperlipemia, insulin.

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Hyperlipemia has been related to the pathogenesis of obesity and diabetes in humans. In particular, it is reported that hyper-free fatty acidemia plays a key role in the pathology of insulin resistance and diabetes [6, 12]. In cats, hypertriglyceridemia is related to a higher serum insulin concentration and lower serum adiponectin concentration, which suggests a close relationship between hyperlipemia and insulin resistance [8]. Because diabetes in cats has been considered to have a pathological condition similar to that in humans [7], it is possible that hyperlipemia also plays a role in the pathology of diabetes in cats.

To evaluate the relationship between hyperlipemia and insulin resistance, an experimental hyperlipemic model has been used in humans and dogs [3, 9]. Generally, a lipid preparation combined with heparin sodium is administered to induce experimental hyperlipemia in studies. However, there are only few studies reporting the effects of intravenous lipid infusion on feline glucose metabolism. In this study, experimental hyperlipemia was induced by lipid infusion in cats, and then the effect of experimental hyperlipemia on insulin sensitivity was evaluated.

Experimental protocols were approved by the Animal Research Committee of Tottori University. Seven cats (3 castrated males and 4 spayed females, mean age of 7.7 ± 1.7, mean body weight of 3.9 ± 0.7 kg) were used in the study. The cats were assessed as healthy on the basis of the results of a physical examination, CBC count and serum biochemical analysis. Each of the cats was examined under two conditions, administration of lipid-heparin and administration of saline with a 2- to 3-week washout period between conditions. After fasting for 15 hr, hyperlipemia was induced according to the method of a previous canine study [3] with slight modification. Briefly, the cats received continuous infusion of 20% fat emulsion (Intralipid, Fresenius Kabi Japan, Tokyo, Japan) containing 16 mU/m*l* of heparin sodium (Fuji Pharma, Tokyo, Japan) or saline into the cephalic vein at a rate of 3 m*l*/kg body weight/hr. Three hours later, serum samples for measurement of the triglyceride (DRI-CHEM, Fuji Medical, Tokyo, Japan) and free fatty acid concentrations (NEFA-HR2, Wako, Tokyo, Japan) were collected, and then general anesthesia was induced with propofol (15 mg/cat, IV) and maintained with isoflurane. Under general anesthesia, a glucose clamp test was carried out according to the method reported in rodents [16] with some modifications. Briefly, regular insulin (Humulin R, Eli Lilly Japan, Kobe, Hyogo, Japan) was administered intravenously at the rate of 10 mU/kg/min, and the blood glucose concentration was measured every 5 to 10 min by use of a portable glucose meter (Medisafe Mini, Terumo, Tokyo, Japan). The optimal infusion rate of insulin was determined in a preparatory experiment (data not shown). Because insulin at the infusion rates reported in humans and rodents failed to effectively decrease the blood glucose concentration in cats in the preparatory experiment, a higher infusion rate was employed in this study. The blood glucose concentration was kept between 100 to 120 mg/d*l* by injection of 50% glucose (Otsuka Pharmaceutical, Tokyo, Japan). Mean glucose infusion rate was calculated from the glucose infusion rate between 90 and 120 min after the start of insulin administration. Variances reported with mean values are the SD, except for the blood glucose concentration and glucose infusion rate, which are reported as means ± SEM. Differences in blood glucose concentration, glucose infusion rate, mean glucose infusion rate and serum triglyceride and free fatty acid concentrations between conditions were analyzed by paired $t$-test. Values of $P$<0.05 were considered significant.

* CORRESPONDENCE TO: NISHII N., Laboratory of Veterinary Pharmacotherapeutics, Faculty of Agriculture, Tottori University, 4–101 Koyama-minami, Tottori 680–5883, Japan.
e-mail: nnishii@muses.tottori-u.ac.jp

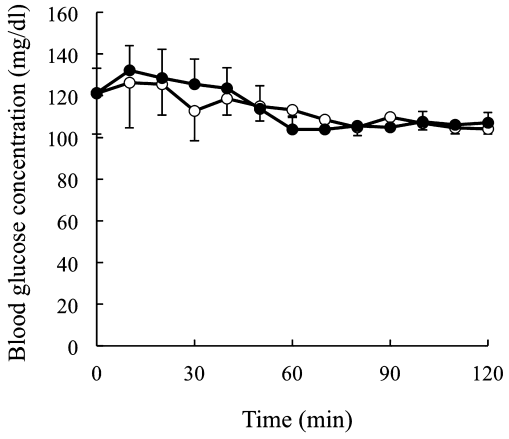


Fig. 1. The blood glucose concentrations of the cats administered lipid-heparin (filled circles) or saline (open circles) during the glucose clamp test. Each circle and bar represents the mean and SEM of 7 cats, respectively. There was no significant difference between two conditions at each time point.

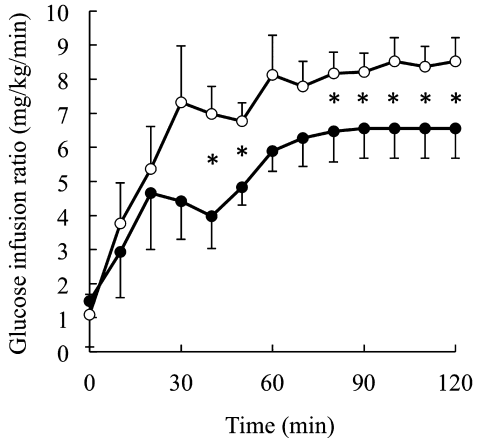


Fig. 2. The glucose infusion ratios of the cats administered lipid-heparin (filled circles) or saline (open circles) during the glucose clamp test. Each circle and bar represents the mean and SEM of 7 cats, respectively. * Within a time point, value differs significantly ($P$<0.05) between two conditions.

Serum triglyceride and free fatty acid concentrations were significantly ($P$<0.05) higher when lipid-heparin was administered (2,894 ± 1,526 mg/d*l* and 4.54 ± 0.70 mEq/*l*, respectively) than when saline was administered (70 ± 42 mg/d*l* and 0.22 ± 0.08 mEq/*l*, respectively). In the glucose clamp test, the blood glucose levels were stabilized 60 min after initiation of insulin administration under both conditions (Fig. 1). There was no difference in blood glucose concentrations between the two conditions. On the other hand, the glucose infusion rate was significantly ($P$<0.05) lower at steady-state when lipid-heparin was administered than when saline was administered (Fig. 2). Also, the mean glucose infusion rate between 90 and 120 min after the start of insulin administration was significantly ($P$<0.05) lower when lipid-heparin was administered (5.80 ± 0.67 mg/kg/min) than when saline was administered (8.52 ± 1.83 mg/kg/min).

Administration of the lipid preparation containing heparin sodium induced marked hypertriglyceridemia and hyper-free fatty acidemia. In previous studies demonstrating experimental hyperlipemia in humans and rodents, the serum free fatty acid concentration reached 1.5 to 8.0 mEq/*l* [1, 2, 13, 14, 18]. The lipid infusion protocol used in the present study achieved comparable hyper-free fatty acidemia in cats. The lipid infusion in the cats induced an approximately 30% decrease in insulin sensitivity evaluated by glucose clamp test. To our knowledge, this is the first study that demonstrates the impairment of insulin sensitivity by lipid infusion in cats. This result shows the importance of hyperlipemia in insulin resistance in cats. The mechanism of insulin resistance induced by lipid infusion is not fully understood yet; however, several hypotheses have been suggested, including inhibition of post receptor insulin signaling [1], alteration of gluconeogenesis at the liver [14] and deficiency in transportation of insulin into skeletal muscles [3]. In contrast, Zini *et al.* [19] reported that lipid infusion for 10 days in cats did not affect insulin sensitivity. In that study, however, hyperlipemia was milder than that in the present study (serum triglyceride concentration of 246 to 574 mg/d*l*). The difference in severity of hyperlipemia might affect the presence or absence of insulin resistance after lipid injection.

Because the lipid preparation is used in intravenous nutrition in cats, the results of the present study may raise a concern about the insulin resistance during intravenous nutrition. In fact, the possibility of a problem in glucose metabolism in human patients undergoing intravenous nutrition has been suggested [17]. Intravenous nutrition has also been associated with hyperglycemia in cats [5, 11]. However, the infusion rate of lipids in this study was two or three times higher than the rate typically used in intravenous nutrition in cats. In addition, heparin sodium, which induces further hyper-free fatty acidemia, is not usually administered to cats under intravenous nutrition. Because lipid injection at a slower infusion rate did not induce insulin resistance in cats [19], further study is needed to conclude whether lipid infusion during intravenous nutrition causes significant insulin resistance in cats.

In this study, the blood glucose concentration was monitored by use of a portable glucose meter. It has been reported that the glucose concentration measured by a portable glucose meter is approximately 20 mg/d*l* lower than that measured by standard methods such as the hexokinase method [15]. Thus, the glucose concentrations shown here would have been higher if the standard method had been used. Although variations in steady-state blood glucose concentration did not affect the accuracy of the glucose clamp test [10], caution should be needed to compare the

results shown here with other results derived from different methods. Another possible limitation of this study is the lack of insulin and gluconeogenesis determinations. Secretion of endogenous insulin and hepatic gluconeogenesis are factors that may complicate the interpretation of glucose clamp test. In theory, endogenous insulin and gluconeogenesis are considerably suppressed by insulin infusion during the glucose clamp test [4], however, it has not yet been confirmed in cats. To preclude the influence of these factors on the glucose clamp test, further study including measurement of blood insulin concentrations, inhibition of endogenous insulin secretion and evaluation of hepatic gluconeogenesis by administration of labeled glucose is needed.

In conclusion, acute infusion of a lipid preparation containing heparin induced significant insulin resistance in cats. The lipid infusion protocol used in the present study is considered to be useful in the feline model of experimental insulin resistance due to hyperlipemia.

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