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Alterations in Adipokines in Feline Hepatic Lipidosis

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Background: Feline hepatic lipidosis (HL) is associated with alterations in lipid and carbohydrate metabolism. The adipokines, adiponectin, and leptin have lipid-lowering and insulin-sensitizing effects.

Hypothesis: Serum concentrations of adiponectin and leptin are altered in feline HL.

Animals: Client-owned cats: 55 healthy and 45 with liver disease.

Methods: Cats with liver disease were categorized as having HL (n = 20), HL and concurrent disease (n = 19), or other liver disease (n = 6), based on clinical signs, laboratory findings, abdominal ultrasound examination as well as liver cytopathology, histopathology, or both. Serum samples were collected and body condition score determined.

Results: Mean serum concentrations of adiponectin were higher in overweight cats with HL (4.5 μg/mL), HL and concurrent disease (4.4 μg/mL), or other liver disease (6.1 μg/mL), as compared with healthy cats (1.5 μg/mL; $P < .001$, $P < .001$, and $P = .04$, respectively). Mean serum concentration of leptin was higher in cats with HL (9.8 ng/mL) or HL and concurrent disease (10.7 ng/mL) than healthy cats (4.9 ng/mL, $P < .001$ and $P < .001$, respectively). Cats with other liver disease had leptin concentration (4.9 ng/mL) similar to healthy cats. Concentrations of adiponectin were correlated with alanine aminotransferase activity ($r = 0.40$, $P = .0069$), and concentrations of leptin were correlated with alkaline phosphatase activity ($r = 0.42$, $P = .0051$) in cats with liver disease.

Conclusions and Clinical Importance: Adipokine concentrations are altered in feline HL. Increased concentrations of adiponectin are related to liver disease, whereas increased concentrations of leptin are specifically related to HL.

Key words: Adiponectin; Insulin; Leptin; Triglyceride.

Feline hepatic lipidosis (HL) is a common and potentially fatal liver disorder. The pathogenesis of this disease still is not completely understood, but its most important identified risk factor is obesity.[1] Obesity may predispose cats to HL mainly by increased availability of free fatty acids (FAs) that can be mobilized from peripheral fat stores during periods of decreased food intake. Additional potential contributing factors include pre-existing insulin resistance,[2] and higher baseline hepatic lipid content[3] related to obesity.

Alterations in lipid and carbohydrate metabolism have been described both in naturally occurring and experimentally induced feline HL.[4–7] Affected cats had increased serum concentrations of free FAs, triglycerides, very low-density lipoproteins (VLDL), and low-density lipoproteins (LDL), as well as decreased insulin concentrations and decreased glucose tolerance.

Adipose tissue plays a key role in energy homeostasis, not only by serving as a storage organ but also by active secretion of adipokines, including adiponectin and leptin. Adiponectin, the most abundant adipokine, exerts a profound insulin-sensitizing effect[8] as well as anti-inflammatory and antiatherosclerotic effects.[9,10] Leptin is an important regulator of adipose tissue mass and has multiple effects to increase insulin sensitivity.[11] Obese and insulin-resistant cats have decreased concentrations of adiponectin, but increased concentrations of leptin, indicating leptin resistance,[2,12–14] similar to findings in other species.[15] Studies in humans and rodents show that adiponectin and leptin also have an important role in lipid metabolism, both directly and through their insulin-sensitizing effects. Most importantly, these adipokines decrease lipid content of non-adipose tissues.[16–18]

Similar to feline HL, human nonalcoholic fatty liver disease (NAFLD) is characterized mainly by hepatic steatosis associated with obesity, and human patients have decreased serum concentrations of adiponectin.[19–22] In addition, adiponectin administration was able to alleviate hepatic steatosis in a rodent model of NAFLD.[23] Therefore, adiponectin has been suggested to play an important role in the pathophysiology of the disease. Development of hepatic steatosis was demonstrated in rodent models of either leptin deficiency or leptin resistance.[16] Studies on leptin in human NAFLD, however, have yielded inconsistent results.[24]

Abbreviations:

ALP	alkaline phosphatase
ALT	alanine aminotrasferase
BCS	body condition score
FA	fatty acid
GLM	general linear model
HL	hepatic lipidosis
LDL	low-density lipoprotein
NAFLD	nonalcoholic fatty liver disease
VLDL	very low-density lipoprotein

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In view of the antisteatotic effect of adiponectin and leptin, we hypothesized that feline HL is associated with dysfunction of these adipokines that would be manifested in altered serum concentrations. To this end, we compared serum concentrations of adiponectin and leptin among cats diagnosed with HL or other liver disease, and healthy cats.

Materials and Methods

Study Design

Forty-five cats with liver disease and 55 healthy cats were enrolled in the study. Client-owned cats with newly diagnosed liver disease were recruited from the Veterinary Teaching Hospitals at Michigan State University (MSU) and at the Hebrew University of Jerusalem (HUJI) and were allocated into 1 of 3 groups: (1) HL (n = 20); (2) HL and concurrent disease (n = 19); and (3) other liver disease (n = 6). Diagnosis of liver disease in all cats was based on clinical signs and laboratory findings, abdominal ultrasonography, and liver cytopathology, histopathology, or both. Exclusion criteria included initiation of enteral feeding or parenteral nutrition, or administration of glucocorticoids before sample collection. The healthy cats were client-owned cats presented to MSU for routine examination. Cats were considered healthy based on the absence of any clinical signs or clinicopathologic findings indicating illness. Cats receiving any dietary supplements or medications were excluded.

Body condition of all cats was evaluated by 2 individuals using body condition scoring (BCS) system on a 1–9 scale,[25] and serum samples were collected after a 12-hour fast. The study was performed in compliance with MSU and HUJI guidelines for research in animals. Informed consent was obtained from all owners.

To determine potential interference of lipemia, hyperbilirubinemia, and hemolysis on measurement of hormones, concentrations of adiponectin, leptin, and insulin were measured in pooled feline serum with added Liposyn,[a] bilirubin,[b] and hemolysate at 6 concentrations ranging from 0 to 1600 mg/dL, 0 to 50 mg/dL, and 0 to 28.3 mg/mL, respectively.

Serum Analysis

Serum was separated immediately after blood collection and frozen at −20°C until analyzed. Serum adiponectin, leptin, and insulin concentrations in all samples were measured at MSU using commercially available assays[cde] as described previously.[26] Serum samples were diluted 1 : 1,111 in preparation for the adiponectin assay. For validation, serial dilutions of feline serum were prepared, and the observed curves were parallel to the standard curves for mouse adiponectin, human leptin, and human insulin standards. The dynamic range and minimal detection limit of the assays was 0.25–8 ng/mL and 15.6 pg/mL in diluted serum for adiponectin, 1–50 ng/mL and 1 ng/mL for leptin, and 36–2,153 pmol/L and 9 pmol/L for insulin. Intra-assay and interassay coefficients of variation were 5% and 7% for adiponectin, 6% and 10% for leptin, and 9% and 10% for insulin. Serum concentrations of glucose, cholesterol, and triglyceride were measured by a spectrophotometric method.[f] Additional biochemistry variables were measured with automated chemistry analyzers.[g]

Data Analysis

Associations between liver disease and the outcome variables (serum concentrations of adiponectin, leptin, insulin, glucose, triglyceride, or cholesterol) were evaluated using general linear model (GLM) including group (healthy cats, cats with HL, cats with HL and concurrent disease, and cats with other liver disease), sex, and site (MSU or HUJI) as factors, and BCS and age as covariates. The additional factors and covariates were included to control for potential confounding of the relationship between liver disease and the outcome variables. The link function and the linear predictor for each of the variables are of the form $E[Y|x] = a + bx$. Y is a natural logarithmic transformation of the measured values and has a normal distribution (as confirmed by Probability–Probability plots) with a mean $(a + bx)$ and constant variances. All interaction effects between group and the additional factors and covariates were assessed. Additional factors, covariates, and interaction terms with significance level of effect $P \geq .1$ were removed from the models. Bonferroni adjustment was applied for pairwise comparisons between each of the liver disease groups and healthy cats. All hypotheses in this study were predefined, and the relationships between the exposure and outcome variables were each of specific interest. Therefore, adjustment for multiple comparisons attributable to testing more than one hypothesis was not applied.

Model Fitting and Posthoc Categorization. The factor "sex" and covariate "age" were removed from the models evaluating the associations between liver disease and all outcome variables because of insignificant effect. The factor "site" was removed from all models except the model evaluating the association between liver disease and insulin. Interaction effects were present between BCS and group in the analyses of the association of liver disease and adiponectin ($P = .004$) or triglyceride ($P = .067$). Because of insufficient statistical power of the study to evaluate the association between liver disease and the outcome variables in separate analyses of each BCS level, categorization into lean (BCS 2–5; n = 41) and overweight (BCS 6–9; n = 59) was used.

Linear correlations between the outcome variables and BCS were evaluated by Spearman correlation coefficient. Measures of additional biochemical variables are expressed as fold increases of the upper reference interval value of serum concentrations or serum enzymes activities to adjust for potential differences between the 2 sites. Linear correlations between the outcome variables and measures of bilirubin, albumin, alanine aminotransferase (ALT), aspartate aminotransferase, and alkaline phosphatase (ALP) were evaluated by Pearson correlation coefficient. Natural log transformation was used for any nonnormally distributed variable (all variables but total bilirubin).

Data were analyzed by a commercially available statistical program.[h] $P < .05$ was considered statistically significant. Results are reported as adjusted geometric mean and 95% confidence interval. Summary data are presented as median and range.

Results

Subjects

The healthy cats group included 25 neutered females and 30 neutered males. The liver disease group included 23 neutered females, 20 neutered males, 1 intact female, and 1 intact male. Age ranged from 1 to 16 years in the healthy cats group and from 2 to 17 in the liver disease group (mean, 4.9 years and 7.6 years, respectively). BCS ranged from 4 to 8 in healthy cats and from 2 to 9 in cats with liver disease (median, 6 in both groups). Frequent breeds among the healthy cats and liver disease groups consisted of domestic short hair (40 and 29), domestic long hair (7 and 9), and mixed breed (3 and 5), respectively. In addition, the healthy cats group included 2 domestic medium hair

cats, 1 Siamese, 1 Birman, and 1 Bengal cat, and the liver disease group included 1 Maine Coon and 1 Siberian cat. Twenty-four cats with liver disease were enrolled at MSU (9 with HL, 10 with HL and concurrent disease, and 5 with other liver disease) and 21 cats at HUJI (11 with HL, 9 with HL and concurrent disease, and 1 with other liver disease). Concurrent diseases in cats with HL included pancreatitis (10), cholangiohepatitis (8), lymphoma (4), and organophosphate toxicity (2). Other liver diseases included cholangiohepatitis (4; 3 enrolled at MSU and 1 at HUJI) and lymphoma (2 enrolled at MSU). Changes in biochemistry variables in cats with liver disease are presented in Table 1.

Interference Testing

Influence of blood components on measurement of leptin, adiponectin, and insulin in pooled feline serum is presented in Figure 1. Percent of expected leptin value ranged from 96 to 110% with added lipid, from 89 to 104% with added bilirubin, and from 93 to 106% with added hemolysate. Percent of expected adiponectin value ranged from 106 to 119% with added lipid, from 91 to 104% with added bilirubin, and from 86 to 108% with added hemolysate. Percent of expected insulin value ranged from 99 to 113% with added lipid, from 96 to 101% with added bilirubin, and from 82 to 101% with added hemolysate.

Associations among BCS and Serum Concentrations of Hormones, Lipids, and Glucose in Healthy Cats and Cats with Liver Disease

Significant moderate[27,28] correlations were found in healthy cats. BCS was significantly positively correlated with serum concentrations of leptin ($r = 0.47$, $P < .001$), insulin ($r = 0.51$, $P < .001$), and triglyceride ($r = 0.48$, $P < .001$), and significantly negatively correlated with serum concentrations of adiponectin ($r = -0.47$, $P < .001$). In cats with liver disease, BCS was significantly positively correlated with serum concentrations of leptin ($r = 0.38$, $P = .013$) only.

Significant moderate correlations were present between adiponectin and leptin ($r = -0.37$, $P = .0053$), adiponectin and insulin ($r = -0.36$, $P = .0067$), and leptin and insulin ($r = 0.42$, $P = .0014$) among healthy cats, but not cats with liver disease. Serum concentrations of triglyceride were significantly correlated with adiponectin in healthy cats ($r = -0.27$, $P = .044$), but not in cats with liver disease, with leptin in cats with liver disease ($r = 0.56$, $P < .001$), but not in healthy cats, and with insulin both in healthy cats ($r = 0.43$, $P = .0011$) and cats with liver disease ($r = 0.35$; $P = .020$).

Serum Concentrations of Hormones, Glucose, and Lipids in Healthy Cats and Cats with Liver Disease

Measured serum concentrations and comparisons of hormones, glucose, and lipids between healthy cats and cats with HL, cats with HL and concurrent disease, or cats with other liver disease are presented in Table 2.

Concentrations of adiponectin were higher in overweight cats with HL, HL and concurrent disease, or other liver disease than in healthy cats ($P < .001$, $P < .001$, or $P = .04$, respectively). No statistically significant differences were identified in lean cats. Concentrations of leptin were significantly higher in cats with HL ($P < .001$) or HL and concurrent disease ($P < .001$) than in healthy cats. Concentrations of leptin in cats with other liver disease were not different from concentration in healthy cats.

Cats with other liver disease had significantly lower concentrations of insulin compared with healthy cats ($P = .011$), whereas there was no difference in concentrations of glucose. Cats with HL or HL and concurrent disease had significantly higher concentrations of glucose compared with healthy cats ($P < .001$ or $P < .001$, respectively), whereas there were no differences in concentrations of insulin.

Concentrations of triglyceride were significantly higher in cats with HL than in healthy cats both in the lean ($P < .001$) and overweight ($P = .016$) groups, whereas there were no differences between cats with

Table 1. Biochemical changes in cats with liver diseases: Median (range) of fold increase in the upper reference interval value and number of cats below and above the reference interval.

	HL (n = 20)			HL and Concurrent Disease (n = 19)			Other Liver Disease (n = 6)		
		No. (%)			No. (%)			No. (%)	
	Fold Increase	Above	Below	Fold Increase	Above	Below	Fold Increase	Above	Below
BUN	0.5 (0.2–1.0)	0 (0)	10 (50)	0.5 (0.2–1.1)	1 (5)	10 (53)	0.4 (0.3–0.9)	0 (0)	2 (33)
Creatinine	0.5 (0.2–1.0)	1 (5)	3 (15)	0.6 (0.4–1.1)	2 (11)	0 (0)	0.6 (0.1–0.8)	0 (0)	1 (17)
Albumin	0.7 (0.5–1.0)	0 (0)	9 (45)	0.7 (0.4–1.0)	0 (0)	9 (47)	0.7 (0.5–0.8)	0 (0)	1 (17)
Total bilirubin	19.3 (0.3–34.1)	18 (90)	0 (0)	14.6 (0.2–36.1)	16 (84)	0 (0)	4.2 (1.5–30.2)	5 (83)	0 (0)
ALP	7.9 (0.1–46.6)	19 (95)	0 (0)	3.5 (0.1–23.7)	17 (89)	0 (0)	0.5 (0.1–6.7)	3 (50)	0 (0)
ALT	1.4 (0.5–10.4)	13 (65)	0 (0)	2.1 (0.4–18.1)	15 (79)	0 (0)	2.1 (1.3–8.9)	6 (100)	0 (0)
AST	1.9 (0.1–6.3)	18 (90)	0 (0)	2.2 (0.1–31.4)	15 (79)	0 (0)	2.6 (0.2–6.1)	4 (67)	0 (0)

BUN, blood urea nitrogen; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

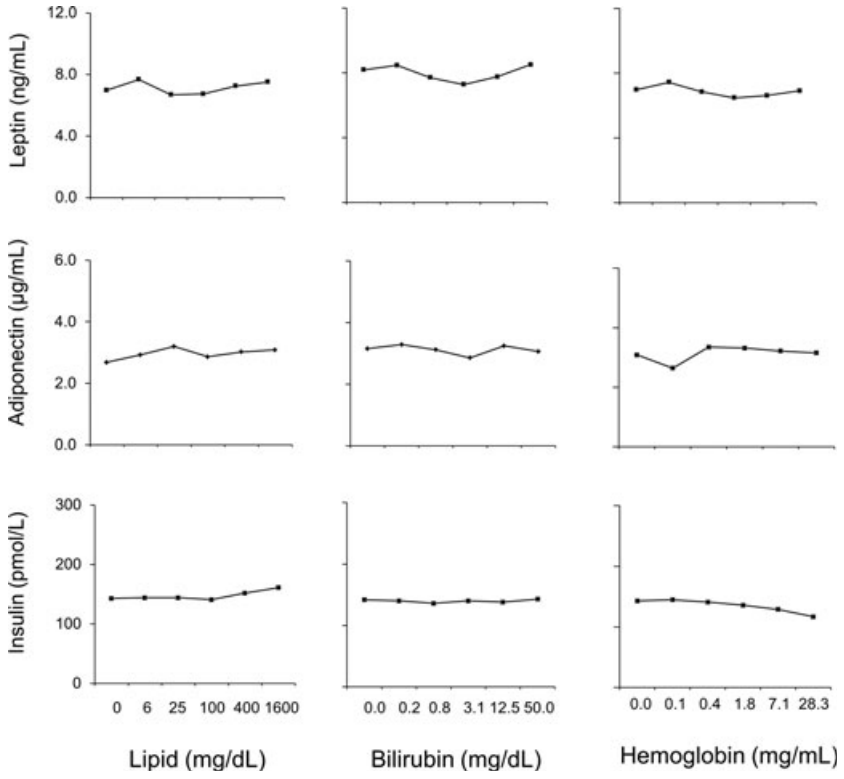

Fig 1. Influence of blood components on hormone assays. Potential interference of blood components on measurement of leptin, adiponectin, and insulin was tested by the addition of lipid, bilirubin, or hemolysate to pooled feline serum.

other liver disease and healthy cats in both groups. Concentrations of triglyceride in cats with HL and concurrent disease were significantly higher compared with healthy cats in the lean ($P = .030$), but not in the overweight group. No significant differences in concentrations of cholesterol between cats with HL, HL and concurrent disease, or other liver disease and healthy cats were present.

Associations between Hormones and Liver Enzyme Activity among Cats with Liver Disease

When all cats with liver disease were grouped together, significant moderate positive correlations were found between serum concentrations of leptin and fold increase in serum ALP activity ($r = 0.42$, $P = .0051$) and between serum concentrations of adiponectin and fold increase in serum ALT activity ($r = 0.40$, $P = .0069$).

Discussion

Serum concentrations of adiponectin were increased in cats with any liver disease, whereas concentrations of leptin were increased in cats with HL (with or without concurrent disease), but not in cats with other liver disease. Cats with HL had increased glucose concentrations and cats with other liver disease had decreased insulin concentrations. Serum concentrations of triglyceride were increased in cats with HL. Bilirubin and lipid appeared to have no effect on measurement of adiponectin, leptin, and insulin, excluding the possibility of spurious alterations in the concentrations of these hormones attributable to hyperbilirubinemia and hyperlipidemia that often accompany liver disease and HL.

The finding of increased serum concentrations of adiponectin in cats with HL is in contrast to its antisteatotic effect, as well as reports of decreased serum adiponectin concentrations in human patients with NAFLD and rodent models of the disease.[19–21,23,29–32] This difference may be related to the dissimilar natural development and progression of feline HL compared with NAFLD in humans. Although the disease is manifested by hepatic lipid accumulation both in humans and cats, human NAFLD commonly is asymptomatic and rarely progresses to advanced liver disease,[33] whereas most cases of feline HL, including the cats in this study, are characterized by evident clinical signs and severe cholestasis at the time of presentation and diagnosis.[1,34]

Pathologic changes associated with liver disease may have an important effect on adiponectin concentration. This is supported by the finding of increased serum adiponectin concentrations in cats with any liver disease and is in agreement with the reported increased serum concentrations of adiponectin in human patients with chronic liver disease accompanied by cholestasis or cirrhosis,[35,36] and in animal models of acute liver injury.[37,38] The increase in

Table 2. Measured (A) and adjusted (B) serum concentrations of hormones, glucose, and lipids in cats with liver diseases and healthy cats.

	Group			
A[a]	Healthy (n = 55)	HL (n = 20)	HL with Concurrent Disease (n = 19)	Other Liver Disease (n = 6)
Adiponectin (μg/mL)				
Lean	2.9 (0.3–7.1)	3.3 (0.9–8.1)	4.3 (2.3–8.2)	6.5 (4.1–7.6)
Overweight	2.0 (0.2–7.6)	4.9 (1.7–8.8)	5.1 (1.5–6.8)	5.3 (4.7–6.0)
Leptin (ng/mL)	4.8 (3.1–13.8)	9.9 (2.4–39.5)	9.2 (5.8–27.0)	3.8 (1.8–28.2)
Insulin (pmol/L)	39 (7–101)	26 (2–90)	22 (6–99)	16 (5–29)
Glucose (mg/dL)	94 (63–157)	135 (48–237)	134 (84–266)	117 (88–190)
Triglyceride (mg/dL)				
Lean	60 (40–116)	159 (129–428)	119 (20–171)	36 (27–68)
Overweight	71 (41–526)	126 (90–720)	123 (96–171)	109 (52–166)
Cholesterol (mg/dL)	133 (68–234)	112 (69–342)	103 (54–364)	126 (67–224)

		Group			
B[b]	Covariates	Healthy (n = 55)	HL (n = 20)	HL with Concurrent Disease (n = 19)	Other Liver Disease (n = 6)
Adiponectin (μg/mL)					
Lean	–	2.4 (1.8–3.4)	3.0 (1.6–5.4)	4.3 (2.8–6.7)	6.0 (2.9–12.5)
Overweight	BCS	1.5 (1.1–1.9)	4.5 (3.0–6.8)[c]	4.4 (2.6–7.6)[c]	6.1 (2.1–17.9)[c]
Leptin (ng/mL)	BCS	4.9 (4.3–5.5)	9.8 (8.0–12.0)[c]	10.7 (8.7–13.2)[c]	4.9 (3.5–7.1)
Insulin (pmol/L)	BCS, site	28 (22–35)	22 (17–28)	25 (19–32)	13 (8–21)[c]
Glucose (mg/dL)	–	100 (92–107)	134 (118–152)[c]	140 (123–159)[c]	122 (97–153)
Triglyceride (mg/dL)					
Lean	–	63 (51–77)	204 (141–294)[c]	98 (75–129)[c]	39 (25–61)
Overweight	BCS	91 (75–109)	152 (113–204)[c]	119 (79–181)	87 (40–190)
Cholesterol (mg/dL)	BCS	136 (123–149)	126 (108–148)	104 (88–123)	115 (86–154)

[a]Measured values (median and range) of serum concentrations.

[b]Adjusted values (geometric mean and 95% confidence interval) of serum concentrations. Associations between group and serum concentrations of hormones, glucose, or lipids were determined using GLM. Additional factors (sex, site) and covariates (BCS, age), as well as interaction terms between group and each of the additional factors/covariates, were evaluated in each model. Factors/covariates with an effect of significance level $P < .1$ were included in each model. Adiponectin and triglyceride were analyzed separately in different body condition groups because of interaction term between BCS and group with $P < .1$.

[c]Indicates significantly different ($P < .05$) from healthy cats following Bonferroni correction. Lean: BCS 2–5, Overweight: BCS 6–9.

adiponectin was more pronounced in overweight than in lean cats with liver disease, leading to a significant interaction between body condition and liver disease. A much lower adiponectin concentration in overweight than in lean healthy cats compared with a similar concentration in cats with liver diseases suggests that body condition is not a strong determinant of adiponectin concentrations in cats with liver disease. This is supported by the finding of a significant moderate negative association between adiponectin and body condition in healthy cats, but not in cats with liver disease. Moreover, similar to findings in other species, serum adiponectin concentrations were inversely related to concentrations of insulin, leptin, and triglyceride in the healthy cats.[2,14,16] The absence of these expected associations among the cats with liver disease further suggests that physiologic mechanisms normally related to circulating adiponectin concentrations may be impaired in liver disease.

Different mechanisms may lead to increased serum concentrations of adiponectin in cats with liver disease. One potential mechanism is decreased excretion of adiponectin in bile because of cholestasis. Increased serum adiponectin concentrations have been demonstrated in biliary diseases in humans and shortly after bile duct ligation in mice.[35] Another possibility is production of adiponectin in the injured liver, potentially as a protective mechanism. Although adiponectin is normally produced predominantly in the adipose tissue, adiponectin protein was detected in endothelial cells in the liver after induction of acute hepatic failure in mice,[37] and adiponectin mRNA expression was detected in the liver of carbon tetrachloride-treated mice.[38] In addition, decreased hepatic function and altered hemodynamics have been shown to result in decreased extraction of adiponectin from the circulation in human patients with cirrhosis.[36] In this study, serum concentrations of adiponectin were directly moderately related to serum ALT activity, but not to serum ALP activity among cats with liver disease. These findings suggest that hepatocellular injury is more important than cholestasis as mechanisms leading to increased circulating adiponectin in these cats.

Specific mechanisms that may contribute to the increase in serum concentrations of adiponectin in cats

with HL also include the presence of hepatic resistance to adiponectin action, as has been suggested in a model of NAFLD in mice.[39] Increases in serum adiponectin also could be a compensatory mechanism to prevent insulin resistance as has been suggested in a recent study that documented increased concentrations of adiponectin after lipid infusion and development of hepatic steatosis in cats.[40]

The increase in serum concentrations of leptin in cats with HL, but not in cats with other liver disease, suggests that this alteration is associated specifically with HL rather than liver disease in general. In addition, serum concentrations of leptin were directly moderately related to serum ALP activity in cats with liver disease. Because ALP activity is an indicator of cholestasis, which may be caused by lipid accumulation in the liver among other causes, this finding suggests a positive association between leptin and hepatic lipid content in cats with liver disease. In agreement with the findings in this study, an early study in humans demonstrated increased serum concentrations of leptin as well as a direct relationship between leptin concentration and liver steatosis in patients with NAFLD.[41] However, studies that followed failed to show a significant change in leptin concentrations.[24,30]

Serum concentrations of leptin were directly related to body condition in cats with liver disease, similar to findings in healthy cats in this study and in previous reports,[2,12,13] suggesting at least partial preservation of the mechanisms that normally determine circulating concentrations of leptin. In view of the antisteatotic effect of leptin, its increased concentrations as well as the positive association with serum triglyceride concentrations in HL may indicate exacerbation of resistance to leptin that occurs in healthy overweight cats. Hepatic leptin resistance may lead to progression of hepatic steatosis despite increased leptin concentrations. Additional factors that may contribute to increase leptin concentrations in human NAFLD include the presence of hepatic inflammation and chronic hyperinsulinemia.[41] However, neither of these pathologic processes is an important feature of the disease in cats and therefore they are unlikely to be involved in the development of hyperleptinemia in feline HL.

Cats with HL had increased serum concentrations of glucose, whereas cats with other liver disease had decreased concentrations of insulin. A previous study[6] reported decreased concentrations of insulin in cats with naturally occurring cholangiohepatitis, similar to the findings in this study, but, decreased concentrations also were shown in cats with HL in that study. In another study,[4] no change in baseline insulin concentration was found in cats with experimentally induced HL, in agreement with this study. In addition, mild hyperglycemia was present in half of the cats with HL or cholangiohepatitis in 1 of these studies.[6]

Unlike the findings of unchanged or lower concentrations of insulin in cats with HL, human NAFLD is associated with increased serum concentrations of insulin because of insulin resistance.[19] This difference may be explained by the fact that most cats are presented after a period of partial or complete anorexia, whereas humans with NAFLD usually are asymptomatic at the time of diagnosis. Normal metabolic adaptation to prolonged fasting in cats with HL may lead to decreased insulin secretion, decreased glucose utilization, and increased hepatic gluconeogenesis.[4] Interestingly, the decrease in concentrations of insulin reached statistical significance only in cats with other liver disease and the increase in concentrations of glucose reached statistical significance in cats with HL, but not in cats with other liver disease. These findings appear to be different from the previous study[6] that found lower insulin concentrations both in cats with HL and cats with cholangiohepatitis, and a similar percentage of cats with hyperglycemia in both groups. However, in both studies, concentrations of insulin were numerically lowest in cats with other liver disease and highest in healthy cats, with cats with HL having intermediate results. The different statistical significance findings between the studies could be caused by sample size effect. The relatively higher concentrations of insulin and glucose found in cats with HL compared with cats with other liver disease in this study may be caused by the presence of obesity-related insulin resistance and increased insulin concentrations in many of these cats before weight loss and development of HL.[4,12]

Serum concentrations of triglyceride in cats with HL were increased compared with healthy cats both in lean and overweight cats, whereas there was no difference in serum concentrations of cholesterol. These findings are in agreement with previous studies in cats with naturally occurring and experimentally induced HL, as well as in humans with NAFLD.[5,6,19] A more pronounced difference between cats with HL alone and healthy cats was present in lean compared with overweight cats, leading to a significant interaction between body condition and liver disease. Increased triglyceride concentration in HL is the result of a combination of increased assembly and secretion of VLDL from the liver because of increased FA mobilization from peripheral adipose tissue, as well as decreased VLDL catabolism by lipoprotein lipase (because of insulin deficiency) and by hepatic lipase (because of altered environment in the liver). Hepatic LDL clearance also may be impaired, although the number of LDL receptors was not decreased.[5,7]

Serum concentrations of triglycerides were inversely moderately related to serum concentration of adiponectin in healthy cats, consistent with the hypolipidemic role of adiponectin and in agreement with findings in humans[16] as well as the reported decreased adiponectin in cats with hypertriglyceridemia.[42] In contrast, no association between serum concentrations of triglyceride and adiponectin was present in cats with liver disease, suggesting impairment of the mechanisms by which adiponectin normally exerts its hypolipidemic effect. Similar to healthy cats, serum concentration of triglyceride was directly related to serum concentrations of insulin in cats with liver disease, suggesting preservation of the association of hyperlipidemia and insulin concentrations in a state of liver disease.

This prospective study describes, for the first time, alterations in circulating concentrations of adiponectin and leptin in cats with HL or other liver disease. An important limitation of the study is the relatively small number of cats with other liver disease. Although this group size was sufficient to reveal statistically significant and potentially biologically and diagnostically important differences in adipokine concentrations, these results should be interpreted with caution, because the 6 cats included in this study may not fully represent the population of cats with other liver disease. In addition, cats were enrolled at 2 sites to achieve sufficient numbers. Potential effects of the different sites were addressed in the analyses and presentation of data summary. Finally, this study was not designed to investigate associations between the degree of pathologic changes in the liver and alterations in circulating concentrations of adipokines. Additional studies are warranted to validate the results of this study and to explore potential associations.

In conclusion, serum concentrations of adiponectin were increased in cats with different types of liver disease, whereas serum concentrations of leptin were increased in cats with HL (either alone or with concurrent disease), but not in cats with other type of liver disease. Future studies are indicated to investigate the utility of measurement of these adipokine concentrations as well the relationship between them as potential noninvasive indicators of HL.

Footnotes

[a] Hospira, Inc, Lake Forest, IL
[b] Sigma-Aldrich, Saint Louis, MO
[c] Mouse/rat Adiponectin ELISA kit-B-Bridge, Mountain View, CA
[d] Multispecies Leptin RIA kit- Millipore, St Charles, MO
[e] Human Insulin RIA kit- Diagnostic Systems Laboratories, Webster, TX
[f] Kodak Ektachem DT60 II Clinical Products Division, Eastman Kodak Co, Rochester, NY
[g] AU640[e] Chemistry-Immuno System, Olympus America, Center Valley, PA and Cobas Mira Chemistry Automated Analyzer, Roche, Rottkreutz, Switzerland
[h] SPSS 19.0 for Windows, SPSS Inc, Chicago, IL

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