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Dyslipidemia in obese cats

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Abstract

Obesity is an important endocrine disorder in cats and is a risk factor for diabetes similar to humans. The goal of this study was to examine the effect of long-term obesity and different diets (high protein, and high carbohydrate supplemented with saturated fatty acids or n-3 polyunsaturated fatty acids) on plasma lipids in the fasted and fed states in 12 lean (LEAN) and 12 obese (OBESE) cats with ultracentrifugation, and nuclear magnetic resonance spectroscopy. OBESE had higher plasma non-esterified fatty acids and triglycerides, as well as very-low-density-lipoproteins (VLDL) consisting primarily of medium-sized particles. The concentration of low-density-lipoproteins (LDL) was comparable between the groups, although OBESE had mostly very small, whereas LEAN had mostly large particles. The concentration of high-density-lipoproteins (HDL) was lower in OBESE and consisted primarily of small particles. Plasma triglycerides, and triglycerides and cholesterol in all lipoproteins increased postprandially. Different diets had little effect on lipids. Our results show that long-term obese cats develop similar lipoprotein changes to humans, yet, hypertension and atherosclerosis have not been described in obese cats. This suggests that dyslipidemia alone is not sufficient to induce hypertension and atherosclerosis. Other anti-atherogenic factors may be present in the obese, dyslipidemic cat.

Keywords: Nuclear magnetic resonance; Ultracentrifugation; Diabetes

1. Introduction

In humans, obesity is one of the characteristics of metabolic syndrome. This syndrome encompasses the coexistence of insulin resistance or glucose intolerance, atherogenic dyslipidemia, and hypertension, among other risk factors for diabetes and cardiovascular disease [1]. Metabolic diseases associated with obesity are known to effect over 50% of the adult population [2]. The lipid and lipoprotein alterations seen in obese individuals include elevated cholesterol, triglycerides, and apolipoprotein B concentrations, as well as higher VLDL, LDL, and lower HDL cholesterol levels [3–5]. It has now been shown in many studies that not the total concentration of a lipoprotein but the lipoprotein subclass concentration and particle size are highly correlated with the development of the co-morbidities of obesity. Increases in large VLDL have been associated with coronary artery disease [6] and are inversely correlated with insulin sensitivity [7]. The addition of triglyceride to HDL enhances the action of hepatic triglyceride lipase resulting in the formation of small dense HDL and accelerated clearance of HDL from the circulation [8]. This, in turn, leads to decreased reverse cholesterol transport from the periphery back to the liver and higher risk

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for developing cardiovascular disease [6,9] and insulin resistance [10]. As in the case of HDL, the addition of triglyceride to LDL renders these particles suitable substrates for hepatic triglyceride lipase, resulting in a subpopulation of small dense LDL particles which are more susceptible to oxidative modification and thus more harmful to the endothelium [11]. The smaller LDL particles also have a lower affinity for the LDL receptor, an increased affinity for cell surface binding sites, and they are more readily taken up by the scavenger receptor [12]. This results in large amounts of cholesterol being taken up by the macrophages in the arterial wall leading to atherosclerosis [13].

Diet can have a significant impact on the risk for atherosclerotic cardiovascular disease in the total human population. Reducing saturated fatty acids in people has been shown to reduce total and LDL cholesterol [14]. Incorporating n-3 polyunsaturated fatty acids into the diet improved glucose and insulin metabolism and dyslipidemia [15]. Contrary to that, high-carbohydrate diets have been documented to cause persistent deterioration of glycemic control, hyperinsulinemia, hypertriglyceridemia and high VLDL cholesterol levels in type 2 diabetics [16]. Lipids and lipoproteins have mostly been measured in the fasted state; however, there is evidence that the postprandial state may be a better predictor for co-morbidities of insulin resistance, primarily atherosclerosis [17–20] and that post-meal hyperlipidemia may actually be more damaging than hyperglycemia [21].

Obesity is now also major health concern for veterinary medicine and parallels trends seen in humans. Approximately 35% of cats in the United States are considered overweight or obese [22], and obesity has been demonstrated as a risk factor for diabetes [23]. Many obese cats progress from obesity to type 2 diabetes because they show alterations in insulin secretion and action similar to those of humans [24–27]. However, they do not show atherosclerosis and hypertension. We hypothesized that this was due to the fact that even in long-term obese and insulin resistant cats, fasted and postprandial lipid and lipoprotein concentrations would not show similarities to those found in obese humans, regardless of diet.

2. Materials and methods

2.1. Animals and diets

Twelve lean (LEAN) neutered adult cats aged 5 ± 2 years and 12 obese (OBESE) neutered adult cats aged 7 ± 1 years of equal gender distribution were used for this study ($p > 0.05$). Obese cats had been obese for over 1 year prior to the beginning of the study. Obesity had been originally induced in the cats by allowing ad libitum food intake, whereas lean cats were fed the amount needed to maintain their body condition.

Cats were maintained at the University of Georgia College of Veterinary Medicine Animal Care Facility under standard colony conditions. They were housed individually and given free access to water. Animal studies were approved by the University of Georgia Animal Care and Use Committee and conducted in accordance with guidelines established by the Animal Welfare Act and the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Cats were determined to be healthy on the basis of results of physical examination and clinical laboratory data. All cats were socialized daily. All cats were fed a commercial diet (Purina Pro-Plan, Nestle Purina, St. Louis, MO) prior to the beginning of this study.

The cats were randomly and evenly allocated to 1 of 3 diets in a Latin Square rotation. The 3 diets include HP, a high protein regimen, SAT, a high carbohydrate/high saturated fatty acid regimen, and 3-PUFA, a high carbohydrate diet enriched in n-3 polyunsaturated fatty acids. The compositions of the 3 experimental diets are listed in Table 1. All diets exceeded AAFCO guidelines for a maintenance diet for adult cats. The cats were fed each diet once daily for 2 months and were tested after the 2 months time period. Food intake was recorded at each feeding, and the cats were weighed once weekly. Food intake was adjusted to maintain each cat's weight within 5% of the weight at the beginning of the study. The weight of the lean cats was 3.5 ± 0.5 kg before and at the end of the study. In the obese cats, it was 7.2 ± 1.2 kg at the beginning and 7.1 ± 1.1 kg at the end of the study ($p > 0.05$). The composition of the diets was known only to one investigator (MW) who was not involved in the experimental execution or data analyses.

2.2. Experimental protocol

Blood samples were collected from the jugular vein and immediately placed into EDTA coated tubes on ice for plasma collection. In addition to samples collected after an overnight fast in LEAN and OBESE, blood samples were also taken from LEAN at the first diet rotation at 3, 6, and 12 h post feeding. All lean cats ate their food within 15 min. After centrifugation of the blood, the plasma was harvested and either used immediately for ultracentrifugation and NMR analysis, or it was stored at $-20\,^{\circ}C$ until assays were performed. Plasma was

Table 1
Diet compositions for diets containing high protein (HP), saturated fatty acids (SAT), or 3-omega polyunsaturated fatty acids (3-PUFA)

Composition	Diet HP	Diet SAT	Diet 3-PUFA
Protein (%)	44.2	33.8	35.1
Fat (%)	14.7	16.9	16.6
CHO (by subtraction) (%)	25.3	32.1	32.8
Fiber (crude) (%)	1.4	1.7	1.5
Ash (%)	8.9	8.2	7.2
Moisture (%)	5.5	7.3	6.8
kcal/g (by calculation)	4.1	4.2	4.2
Fatty acid analysis	% fat		
14:0	1.6	2.01	2.61
14:1	0.4	0.4	0.3
16:0	21.3	22.3	21.4
16:1n-7	3.6	3.7	4.4
18:0	10	11.8	10.2
18:1n-9	32.9	34.2	30.3
18:1n-7	1.54	1.53	1.77
18:1n-9 T	2.8	3.4	3.4
18:2n-6	18.5	13.5	12.8
20:0	0.2	0.2	0.2
18:3n-3	1.0	0.8	0.9
20:2n-6	<0.1	<0.1	0.2
20:3n-6	0.1	0.10	0.1
20:4n-6	0.5	0.4	0.5
24:0	0.12	<0.1	<0.1
20:5n-3	<0.1	<0.1	1.9
22:5n-3	<0.1	<0.1	0.4
22:6n-3	<0.1	<0.1	1.5
Total	92.5	91.8	89.9

Ingredients: Whole chicken, poultry by product meal, brewers rice, corn gluten meal, soybean meal (dehulled), whole corn (yellow), beef tallow, menhaden fish oil, corn bran, palatability coating, phosphoric acid, potassium chloride, mineral premix, NaCl, L-lysine, taurine, choline chloride.

used for separating lipoprotein fractions with a single isopycnic density gradient ultracentrifugation method as previously described [28]. Briefly, the plasma was prestained with Sudan black and ethylene glycol solution to enhance observation of the lipoprotein fractions. A swinging bucket rotor (Beckman S 28.1, Fullerton, CA) was used to centrifuge samples at 150,000 × *g* for 31 h at 20 °C. The fractions were collected from the meniscus downward. The density of each fraction was determined by weighing each fraction and measuring the volume of each fraction. In plasma and also in each fraction, cholesterol (Cholesterol LiquiColor; Stanbio, Boerne, TX), triglycerides (Serum Triglyceride; Sigma, Saint Louis, MO), and phospholipids (Phospholipids B; Wako Chemicals, Richmond, VA) were measured. Protein content was determined by the Bradford method [29]. Plasma non-esterified fatty acids (NEFAs) were measured by the use of an enzymatic test kit (NEFA-C; Wako Chemicals, Richmond, VA).

Plasma lipoprotein subclasses and particle size were determined by a commercially available NMR spectroscopy assay (Liposcience, Raleigh, NC). The analysis has been described in detail [30,31]. The proton NMR spectra of aliquots (0.25 mL) were acquired in duplicate at 47 °C with a 400-MHz NMR analyzer. Spectral deconvolution was performed to determine the amplitudes of the lipid methyl group signals broadcast by particles of different size, and these amplitudes were used to quantify the concentrations of the various lipoprotein subclasses. Plasma glucose measurements were performed using a colorimetric glucose oxidase method (Glucose Assay; DCL, Oxford, CT). Plasma insulin concentrations were measured as previously described [32].

Measurement of body mass index (BMI; expressed in kg/m^2) was performed as described [33]. The BMI was calculated by use of the following formula:

$$\mathrm{BMI} = \frac{\text{body weight(kg)}}{\text{body length(m)} \times \text{height(m)}}$$

where length was the distance from the point of the shoulder to the tuber ischium, and height was the distance from the point of the shoulder through the point of the elbow to the proximal boundary of the central metacarpal pad. All measurements were performed by the same person (SK).

2.3. Statistical analysis

Data were tested for normality and multinormality of repeated measures on diet. If the data were multinormal then repeated measures analysis of variance was used. If the data were not multinormal, the analysis was carried out using a mixed models analysis. When diet, or diet × weight, differed significantly, pairwise comparisons were made using Bonferroni adjustment of *p*-values. Analyses were carried out using Systat 12.0 (Systat Inc., Richmond, CA). Data are expressed as means ± S.D. unless otherwise stated. Values of $p < 0.05$ were considered significant.

3. Results

The weight, BMI, girth, and intake of the cats are shown in Table 2. Lean cats consumed significantly more energy per body weight than the OBESE ($p < 0.001$) but there was no diet or gender difference ($p > 0.05$). Weight, BMI, and girth were significantly lower in LEAN than in OBESE ($p < 0.001$).

Table 2
Mean (±S.D.) values for body weight (kg), body mass index (kg/m^2), girth (cm), and food intake (kcal/kg) in lean ($n = 12$, 6 male and 6 female) and obese ($n = 12$, 6 male and 6 female) cats fed 3 different diets

	Measurements			
	Weight (kg)	BMI (kg/m^2)	Girth (cm)	Food intake (kcal/kg)
Lean	3.5 ± 0.5^{a}	32.3 ± 3.9^{b}	35 ± 2.8^{c}	53.8 ± 9.4^{d}
Obese	7.1 ± 1.1^{a}	62.3 ± 8.6^{b}	54.6 ± 4.6^{c}	39.1 ± 7.1^{d}

The results were combined because there were no differences among diets. Superscript letters (a–d) denote significant difference ($p < 0.0001$) between lean and obese groups.

3.1. Plasma fasting lipid concentrations

There was no effect of diet on any of the fasting variables (individual data not shown), and results from all 3 diets for each group were therefore combined (Table 3). Cholesterol concentrations (mg/dL) were not significantly different between LEAN and OBESE ($p > 0.05$). They ranged from 106 to 224 in LEAN, and from 90 to 213 in OBESE. Triglyceride (mg/dL) and protein concentrations (mg/dL) were significantly lower in LEAN than in OBESE, whereas LEAN had significantly higher phospholipid concentrations than OBESE. NEFA concentrations were significantly lower in LEAN than OBESE.

Table 3
Mean (±S.D.) concentration of lipids, protein, and NEFA in fasting plasma and plasma lipoprotein fractions in lean ($n = 12$, 6 male and 6 female) and obese ($n = 12$, 6 male and 6 female) cats fed 3 different diets

Measurements	Plasma	VLDL	HDL_3
Lean cats			
Cholesterol (mg/dL)	132 ± 36	3 ± 3^{e}	49 ± 11
Triglyceride (mg/dL)	21 ± 8^{a}	7 ± 7^{f}	3 ± 2
Phospholipids (mg/dL)	261 ± 40^{b}	3 ± 3^{g}	82 ± 21
Protein (mg/dL)	5819 ± 734^{c}	1 ± 1^{h}	116 ± 36
Total LP mass (mg/dL)	–	13 ± 7^{i}	248 ± 45
NEFA (mEq/L)	0.40 ± 0.19^{d}	–	–
Obese cats			
Cholesterol (mg/dL)	139 ± 28	5 ± 2.9^{e}	45 ± 11
Triglyceride (mg/dL)	48 ± 19^{a}	33 ± 21^{f}	3 ± 2
Phospholipids (mg/dL)	222 ± 46^{b}	10 ± 5^{g}	73 ± 18
Protein (mg/dL)	6301 ± 867^{c}	5 ± 3^{h}	110 ± 31
Total LP mass (mg/dL)	–	52 ± 20^{i}	229 ± 43
NEFA (mEq/L)	0.56 ± 0.12^{d}	–	–

The results for each diet were combined for each group for plasma concentrations, VLDL and HDL_3 fractions because there were no differences among diets. Diet differences were noted in the LDL and HDL_2 fractions which are shown in Tables 4 and 5, respectively. Values with the same superscript letter differ significantly. $^{a,f,g,i}p < 0.001$; $^{b,d}p < 0.004$; $^{c}p < 0.03$; $^{h}p < 0.003$.

Table 4
Mean (±S.D.) concentration of lipids and protein in the fasting LDL fraction in lean and obese (each $n = 12$, 6 male and 6 female) cats fed diets containing high protein (HP), saturated fatty acids (SAT), or n-3 polyunsaturated fatty acids (3-PUFA)

Measurements	HP	SAT	3-PUFA
Lean cats			
Cholesterol (mg/dL)	25 ± 8^{b}	24 ± 12^{a}	$33 \pm 13^{a,b}$
Triglyceride (mg/dL)	4 ± 2^{d}	5 ± 2^{c}	$8 \pm 4^{c,d}$
Phospholipids (mg/dL)	23 ± 6^{f}	21 ± 10^{e}	34 ± 16^{ef}
Protein (mg/dL)	12 ± 3^{h}	11 ± 5^{g}	$16 \pm 6^{g,h}$
Total LP mass (mg/dL)	63 ± 19^{j}	61 ± 27^{i}	91 ± 34^{ij}

	Combined diets
Obese cats	
Cholesterol (mg/dL)	24 ± 9
Triglyceride (mg/dL)	4 ± 5
Phospholipids (mg/dL)	23 ± 10
Protein (mg/dL)	13 ± 4
Total LP mass (mg/dL)	63 ± 22

Results were combined for obese cats because there were no diet differences. Values with the same superscript are significantly different. $^{a,d}p < 0.01$; $^{c}p < 0.05$; $^{e,g,i}p < 0.001$; $^{f,h}p < 0.03$; $^{f,j}p < 0.02$; $^{b}p = 0.056$.

3.2. Concentration of lipid and protein in plasma lipoprotein fractions from fasted cats

3.2.1. VLDL fraction

Body condition but not diet affected the concentrations of VLDL components (Table 3). Cholesterol, triglycerides, phospholipids, and protein were all lower in LEAN than in OBESE causing a 4-fold difference in the lipoprotein mass between LEAN and OBESE. However, on a percent weight basis, VLDL particles in LEAN contained approximately twice the amount of cholesterol than in OBESE ($p < 0.001$), whereas triglycerides were approximately 20% lower ($p < 0.02$).

3.2.2. LDL fraction

The components of the LDL fraction were not different between LEAN and OBESE. However, LEAN fed 3-PUFA had higher values of cholesterol, triglycerides, phospholipids, and protein than lean cats fed either SAT, or HP, causing an increase in the lipoprotein mass by 50%, whereas there was no difference between lean cats fed diet HP and SAT (Table 4).

3.2.3. HDL_2 and HDL_3 fraction

HDL_2 cholesterol, phospholipid, and total lipoprotein mass were significantly higher in the LEAN than

Table 5
Mean (±S.D.) concentration of lipids and protein in fasting HDL_2 fractions in lean ($n = 12$, 6 male and 6 female) and obese ($n = 12$, 6 male and 6 female) cats fed 3 different diets

Measurements	HP	SAT	3-PUFA
Lean cats			
Cholesterol (mg/dL)	67 ± 19[a]	59 ± 17[b]	61 ± 11[c]
Triglyceride (mg/dL)	3 ± 3	3 ± 3	3 ± 3
Phospholipids (mg/dL)	93 ± 28	89 ± 24	86 ± 17[d]
Protein (mg/dL)	118 ± 40	112 ± 35	108 ± 24
Total LP mass (mg/dL)	273 ± 87	263 ± 75	258 ± 50[e]
Obese cats			
Cholesterol (mg/dL)	58 ± 9[a]	52 ± 13[b]	48 ± 11[c]
Triglyceride (mg/dL)	4 ± 3	3 ± 1	3 ± 1
Phospholipids (mg/dL)	78 ± 16	72 ± 15	64 ± 18[d]
Protein (mg/dL)	110 ± 27	95 ± 21	90 ± 32
Total LP mass (mg/dL)	250 ± 49	221 ± 46	205 ± 64[e]

Values with the same superscript letter differ significantly. [a–c]$p < 0.03$; [d]$p < 0.01$; [e]$p < 0.04$.

the OBESE fed 3-n PUFA. Cholesterol was also significantly higher in LEAN cats fed HP and SAT compared to OBESE (Table 5). HDL_3 concentrations were not significantly different between LEAN and OBESE groups (Table 3).

3.3. Particle size and concentration determined by NMR in fasted cats

In OBESE and LEAN, VLDL particles had the largest size, followed by LDL, and HDL particles (Table 6).

In OBESE, medium VLDL particles were seen in all cats, whereas large VLDL particles were only seen in cats fed HP and SAT. The concentration of medium particles was similar among diets (average 5.6 ± 5.3 nmol/L; range of p-values: 0.169–0.492) and higher than that of large particles (average 2.3 ± 2.4 nmol/L; $p = 0.01$). In LEAN, large particles and medium particles were measured, and the concentration was not different (not shown).

Table 6
Mean (±S.D.) size of lipoprotein particles measured with NMR in 12 lean and 12 obese cats after an overnight fast

Measurements	Combined diets	
	Lean	Obese
VLDL particle size (nm)	92.5 ± 44.7[a]	58.2 ± 9.5[a]
LDL particle size (nm)	22.4 ± 0.7[b]	21.5 ± 1.3[b]
HDL particle size (nm)	9.7 ± 0.2[c]	9.4 ± 0.2[c]

Lean VLDL particle size was determined in 8 cats. Values with the same superscript differ significantly. [a,c]$p < 0.001$; [b]$p < 0.01$

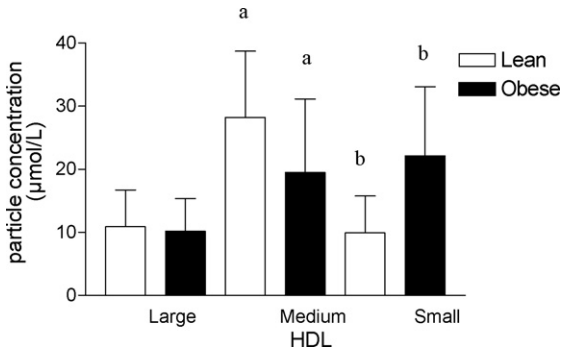

Fig. 1. NMR determined HDL particle concentrations (μmol/L) in lean and obese cats ($n = 24$, 12 L and 12 OB). (A) and (B) denote significant difference ($p = 0.01$) between lean and obese particle concentrations.

The LDL particles could be separated into large, medium small, and very small particles. In LEAN only large particles were detected and their concentration was significantly different than that of OBESE (345.4 ± 52.6 nmol/L and 198.3 ± 40.9 nmol/L, respectively; $p < 0.04$). Most of the particles in OBESE were very small (440.1 ± 273.4 nmol/L), followed by large particles, and medium small particles (115.4 ± 64.7 nmol/L). The concentration of large particles was 50% lower in OBESE fed 3-PUFA compared to the other 2 diets ($p < 0.03$), and there was no effect of diet on the concentration of medium small and very small particles. The concentration of large particles was also significantly lower in overnight fasted LEAN fed 3-PUFA compared to LEAN fed HP and SAT ($p < 0.02$) and postprandial samples at 12 h compared to HP and SAT ($p < 0.02$).

HDL particles were separated into large, medium, and small particles. Concentrations of large HDL particles in samples from fasted cats were not significantly different between LEAN and OBESE ($p > 0.05$). However, medium HDL particle concentrations were significantly higher in LEAN than OBESE, whereas concentrations of small HDL particles were significantly lower in LEAN than in OBESE (Fig. 1). There were no dietary effects.

3.4. Postprandial lipoprotein and NEFA concentrations in lean cats

There was no change in NEFA concentrations after feeding (not shown). The concentrations of plasma cholesterol, and triglycerides before and after food intake are shown in Table 7. The changes in cholesterol concentrations and triglyceride concentrations in lipoprotein fractions at baseline and post-feeding are shown in Figs. 2 and 3, respectively. Feeding increased

Table 7
Mean (±S.D.) concentration of lipids and protein in fasting and postprandial plasma samples in lean cats ($n = 12$, 6 males and 6 females)

Measurements	All diets			
	Baseline	3 h	6 h	12 h
Lean cats (6 males and 6 females)				
Cholesterol (mg/dL)	140 ± 25	145 ± 24	146 ± 21	139 ± 27
Triglyceride (mg/dL)	22 ± 5[ab]	35 ± 13[ac]	34 ± 12[b,d]	23 ± 7[cd]

Values with the same superscript differ significantly. [a–d]$p < 0.001$.

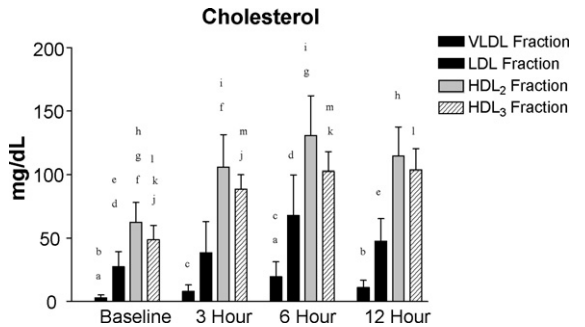

Fig. 2. Fasting and post-feeding cholesterol concentrations (mean ± S.D.) for lipoprotein fractions after ultracentrifugation in 12 lean cats. Values with the same superscript letter vary significantly ([a,f–h,j–l]$p < 0.001$; [c,d]$p < 0.002$ [b,m]$p < 0.02$; [e,i]$p < 0.03$).

the particle concentration only in the VLDL but not in any other lipoprotein fractions post feeding. The VLDL particle concentration was significantly higher at 3 h post-feeding compared to fasting (1.53 ± 1.36 nmol/L vs 0.71 ± 0.64 nmol/L; $p < 0.03$).

3.5. Plasma glucose and insulin concentrations

Diet and body condition had no effect on fasting and postprandial glucose concentrations ($p > 0.05$ for diet and body condition). Glucose values ranged from 63 to 115 mg/dL. Diet also had no effect on fasting insulin concentrations (data not shown). However, fasting insulin concentrations (pmol/L) were significantly lower in LEAN than OBESE (131 ± 68 and 270 ± 100, respectively; $p < 0.002$).

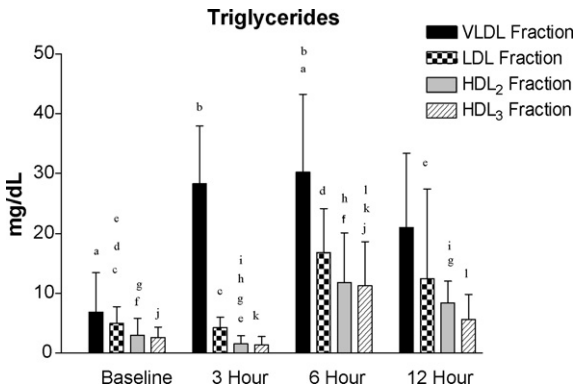

Fig. 3. Fasting and post-feeding triglyceride concentrations (mean ± S.D.) for plasma and lipoprotein fractions after ultracentrifugation in 12 lean cats. Values with the same superscript letter vary significantly ([a]$p < 0.04$; [b]$p < 0.03$; [c,d,f,h]$p < 0.001$; [e]$p < 0.003$; [g]$p < 0.05$; [i]$p < 0.02$).

4. Discussion

Obesity is a risk factor for diabetes mellitus in cats [24,25]. The type of diabetes that develops in obese cats is similar to human type 2 diabetes mellitus (T2DM) including the formation of islet amyloid, a hallmark of the disease in humans. Obesity and diabetes are associated with marked alterations in lipid metabolism in people, and many of the health problems associated with diabetes are, in fact, arising from abnormalities in lipoproteins. Dyslipidemia of diabetes in people is characterized by high levels of triglyceride (TG)-rich VLDL, low levels of HDL cholesterol, small, dense LDL, and impaired and prolonged postprandial hyperlipidemia [10,34]. These abnormalities are present for years before T2DM is diagnosed clinically.

We have previously shown that newly obese cats show an increase in HDL [28] rather than a decrease as seen in people. As neither atherosclerosis nor hypertension has been reported in the obese and diabetic cats, we suggested that this increase in HDL conveys some protective benefit. Data from this study, however, shows that lipoproteins in cats that have been obese long-term show many of the abnormalities described in human obesity. The lipoprotein changes seen in obese people are clearly associated with insulin resistance [35]. While we have not directly evaluated insulin resistance in this group of cats, we have previously shown using the euglycemic hyperinsulinemic clamp that an increase in body weight by 1 kg leads to a decrease in insulin sensitivity by approximately 30% [36,37]. We also have indirect evidence that the obese cats of this study were insulin resistant based on their normal fasting glucose but high insulin concentration.

Similar to obese human patients, obese cats showed an increase in plasma triglycerides and non-esterified fatty acids. It is thought that the increased amount of non-esterified fatty acids being shuttled to the liver is one of the factors involved in increased production and secretion of VLDL [38,39] increasing plasma VLDL concentrations. Despite the high VLDL concentrations long-term obese cats had no change in baseline LDL concentrations indicating either that VLDL was metabolized rapidly and LDL clearance was increased to maintain normal levels or that VLDL was increased because of a lower lipoprotein lipase activity leading to lower hydrolysis of triglycerides from VLDL. Tracer studies would have to be employed to study this further. An increase in VLDL was also seen in newly obese cats [28]. Obese people usually have high VLDL and LDL concentrations, although maintenance of LDL at normal levels has been seen in Pima Indians, and it was suggested that it was due to rapid catabolism of LDL and increased metabolism of VLDL without conversion to LDL [40]. The fact that LDL is normal in Pima Indians who have a high prevalence of diabetes and obesity may explain their lower occurrence of cardiovascular disease [41]. Similar to obese patients with insulin resistance, the obese cats of this study had high VLDL triglycerides. The increased triglyceride concentrations in plasma are largely due to this increase in triglycerides in the VLDL fraction which in long-term obese cats was on average 500% higher, whereas it was approximately 180% higher in newly obese cats compared to lean cats. The increase is probably caused by the decrease in lipoprotein lipase activity which we have documented in obese cats [42]. It has been shown in humans that heparin-releasable lipoprotein lipase is a determinant of triglyceridemia and is negatively correlated with insulin resistance [43]. The overproduction of VLDL in cats was associated with an increase in the VLDL particle number. The particles were of large and medium size which, in people, has been associated with cardiovascular disease [6]. Especially large triglyceride-enriched VLDL can bind to LDL receptors and lead to the formation of cholesterol-rich foam cells [44–46]. Large VLDL particles are linked with small LDL and HDL particles [47,48], and it has been suggested that the high triglyceride contents of large VLDL is the major predictor of LDL size in type 2 diabetics [39]. Increased levels of small, dense LDL have been shown to be strongly associated with coronary artery disease risk in people [49–52]. Overproduction of VLDL has been associated with decreased expression of peroxisome proliferator-activated receptor α (PPARα). PPARα is involved in adipocyte mitochondrial biogenesis and the upregulation of genes involved in fatty acid oxidation [53], and is low in obese cats [54].

Obese cats had an almost 3-fold higher concentration of very small and medium small LDL particles than large particles, while only large particles were detected in lean cats. It was not surprising to find only large particles in lean cats. It was interesting that the 3-PUFA fed cats had a lower LDL particle concentration compared to cats fed the other two diets because we have previously shown beneficial effects of n-3 polyunsaturated fatty acids on insulin sensitivity [55]. The effect of diet on lipoproteins is controversial. It has been shown by some investigators that saturated fatty acids increase the size of LDL particles [56], while others have shown that saturated fatty acids decrease and polyunsaturated fatty acids increase their size [57]. Others have only seen a decrease in LDL particle size with eicosapentaenoic acid but not docosahexaenoic acid [58] or have seen little influence of dietary fatty acids [59]. Beneficial effects on LDL particle number have been shown when high-fiber foods such as oat bran were consumed [60].

Small HDL particles have also been associated with cardiovascular disease. Similar to obese people, small particle concentrations were significantly higher in obese cats. It has been proposed by Taskinen [39] that small HDL is a new component of the metabolic syndrome and associated with atherosclerosis [6]. In particular, it has been found that obesity is associated with a decrease of large HDL_2 and an increase of small HDL_3 particles [61,62]. Our data suggests that small HDL particles are a feature of obesity in cats but not associated with atherosclerosis. While we have not examined if the cats of this study had atherosclerosis, atherosclerosis has not been described in obese cats, and we also have yet to find atherosclerotic lesions in obese cats that we examined (data unpublished). An interesting finding of this study was that long-term obesity leads to a decrease in HDL_2 but not HDL_3 cholesterol. In newly obese cats, an increase in both HDL_2 and HDL_3 cholesterol was seen [28]. In people, a decrease in HDL cholesterol is usually associated with triglyceride enrichment; however, this was not observed in this study. Triglyceride enrichment leads to enhanced clearance of HDL [63]. It is not known if this might also be the case in cats. Recently, postprandial lipemia has received great attention. Postprandial lipid measurements unmask abnormalities earlier than lipid measurements in the fasting state and it is currently thought that postprandial lipemia creates metabolic perturbations which predict cardiovascular disease [64]. The postprandial phase is regarded as a major determinant of oxidative stress [65]. During this phase, VLDL and LDL are enriched with peroxidation-prone

triglycerides. Postprandial remnant-like particles have been shown to increase cellular oxidant content and impair endothelial function [66]. Insulin resistant subjects have a much greater postprandial lipemia than healthy subjects exposing them to greater risk of lipid peroxidation [17,21]. We only measured a time course of postprandial lipids in lean cats but not in obese cats because obese cats do not eat all of their calories within a short enough time to allow a comparison with lean cats but eat throughout the day. Eating increased triglycerides in plasma on average by 50% and in lipoprotein fractions 3–5.5-fold during the first 6 h in lean cats. This is similar to the time course seen in people after ingestion of an oral fat load [67]. It is usually thought that insulin resistance causes hypertriglyceridemia [68]. We assume that insulin resistance causes lipoprotein changes in cats because we have demonstrated that cats have high insulin levels consistent with insulin resistance before they show other abnormalities, including glucose intolerance [55].

In summary, obesity but not diet was the main factor in lipid abnormalities that were seen in long-term obese cats. While the lipoprotein changes were similar to those seen in humans with metabolic syndrome, the fact that atherosclerosis and cardiovascular disease is not a feature of obesity and diabetes in cats suggests that additional factors are important in their pathogenesis.

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