Lipoprotein profiles in Miniature Schnauzers with hypertriglyceridemia

Authors: Troy Bunn[1], Kathrin Langner, Susan Foster, Douglas Hayward, Gretta Howard, Saverio Paltrinieri[2], Alessia Giordano[2], Gabriele Rossi[1]

Institutions:

[1] Murdoch University, Perth, Western Australia (TB, GR)

[2] Dipartimento di medicina veterinaria e scienze animali, Università degli studi di Milano via dell'università 6, 26900 Lodi (SP, AG)

Corresponding author:

Dr. Troy Bunn

Western Australian Veterinary Emergency and Specialty, 08 9412 5700,

troy@wavets.com.au

Abstract

Background

Miniature Schnauzers are predisposed to both primary hypertriglyceridemia (HTG) and hypercortisolism (HC). No study has compared the lipoprotein profiles of Miniature Schnauzers with primary HTG to those with HC (with or without HTG).

Objectives

To measure and compare cholesterol and triglycerides concentrations and lipoprotein fractions in Miniature Schnauzers with and without HTG.

Materials and methods

Miniature Schnauzers with HTG (primary or secondary to HC) and normotriglyceridemia (with or without HC) were included. Lipoprotein fractions were assessed by lipoprotein electrophoresis and compared for each group.

Results

Fifty-one plasma samples were analysed. There were 26 dogs with normotriglyceridemia (19 healthy and 7 with HC) and 25 dogs with HTG (19 with HC and 7 with primary HTG). Hypertriglyceridemic dogs (primary HTG and HC-HTG) had significantly higher concentrations of triglycerides when compared to the normotriglyceridaemic dogs (healthy or HC-NTG). Dogs with primary HTG or HC-HTG had significantly higher cholesterol than healthy dogs. There was a significantly lower HDL percentage in HTG dogs with HC than in HC without HTG. LDL percentage was significantly higher in primary HTG and HC-HTG than in HC.

Conclusions

It was not possible to distinguish dogs with primary HTG from dogs with HC-HTG using lipoprotein electrophoresis fractions.

Introduction

Lipoproteins are carrier molecules in the blood which aid in transport of various lipids[1–3]. The major classes of lipoprotein fractions include chylomicrons, very-low density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL)[2–4]. These lipoprotein fractions have been rigorously studied in human medicine and help aid in predicting and investigating diseases in humans, especially coronary heart disease[5,6]. Lipoprotein profiling in dogs is by contrast, in its

infancy although several studies have been performed reporting the profiles in both health and disease[7-17].

Miniature Schnauzers (MS) are thought to have a genetic alteration in lipid metabolism, predisposing them to fasting hypertriglyceridemia (HTG), but has yet to be identified[16]. Reduced lipoprotein lipase (LPL) activity has been reported in dogs with idiopathic hyperlipemia, suggesting LPL deficiency as a possible cause or predisposing factor[18]. Variants in the coding exons of apolipoprotein C2 (APOC2) gene, the second most common cause of familial chylomicronemia in humans, have been ruled out in MS with idiopathic hypertriglyceridemia in North America[19,20]. Primary HTG is a common condition in MS in the United States with a reported prevalence of 32.8%[21]. To date, an underlying mechanism for the HTG remains unknown however a study utilising both ultracentrifugation and electrophoresis to investigate the lipoprotein profiles of MSwith primary HTG found that affected dogs had increased VLDLs, with or without an accompanying chylomicronemia[16]. Another more recent study used a novel lipoprotein density profiling method to compare lipoprotein profiles in a population of healthy MS with profiles from non-Miniature Schnauzer dogs and MS with primary HTG[13]. The abundance of major lipoprotein classes in MS with normal triglyceride concentration were similar to healthy dogs of various breeds. However, most MS with normal triglycerides showed some distinct differences in some lipoprotein fractions: triglyceride-reach lipoprotein (TRL) mainly constituted by chylomicrons are more prominent in MS than other breeds; on the contrary, other breeds had more prominent LDL. Consistent with the earlier study, the lipoprotein profile of MS with primary HTG was characterised by an increase in the TRL and a decrease proportion of LDL than MS with normal triglyceride

concentration. [13].It was also found that MS may have lower cholesterol concentrations than dogs of other breeds. The clinical importance of these differences is unknown; low-fat diet in MS with hyperlipemia significantly reduces triglycerides and cholesterol as well as TRL and LDL[22]. Other than primary hyperlipemia, the most common disorders of lipoprotein metabolism in dogs are secondary to other diseases such as diabetes mellitus, hypothyroidism, hypercortisolism and pancreatitis[3]. In previous studies evaluating the lipoprotein profile of MS the main limitation was a lack of rigorous exclusion of all secondary causes for fasting HTG; thus, the diagnosis of primary HTG was presumptive. While thyroid and pancreatitis were definitively ruled out with laboratory tests, hypercortisolism (HC) was excluded based on the absence of clinical signs, without performing functional tests. A metabolomic study performed in dogs with hypercortisolism demonstrated hypercholesterolemia, hypertriglyceridemia, increased VLDL and total fatty acids[23]. To the authors knowledge, the differences of lipoprotein patterns in MS with primary and secondary hypertriglyceridemia have not been investigated.

The purpose of this research was to compare cholesterol, triglycerides and lipoprotein profiles in healthy Miniature Schnauzers and compare with 1) MS with primary HTG 2) MS with HTG secondary to hypercortisolism; 3) MS with hypercortisolism without HTG.

Materials and methods

Animals and sample population

All samples were collected as part of another study evaluating HTG in Australian Miniature Schnauzers with an approved Animal Ethics Permit from Murdoch University (R3174/19). As part of this other study, Miniature Schnauzers of all ages, sex and neuter status were recruited across Australia. Exclusion criteria included previously diagnosed hypercortisolism, diabetes mellitus or hypothyroidism, treatment with drugs known to affect lipid metabolism (e.g., corticosteroids and phenobarbitone) within the last 2 months, medical illness requiring veterinary attention in the last 4 weeks and current lactation or pregnancy. Included dogs had general health assessed by clinical examination and had fasting serum triglycerides measured after a 15-hour fast. For Miniature Schnauzers with fasting HTG, to distinguish between primary and secondary hyperlipemia, dogs underwent further investigations including the measurement of a systolic blood pressure, complete blood count (CBC) with blood smear evaluation, serum biochemistry including 1,2-o-dilauryl-rac-glycero glutaric acid-(6'-methylresorufin) ester (DGGR) lipase activity and urinalysis. Thyroid function was assessed by the measurement of total T4 and if outside the reference interval, a concurrent canine thyroid stimulating hormone (cTSH) measurement was also performed. Similarly, to assess the adrenal function a standard low dose dexamethasone suppression test (LDST) was performed and if the results were negative or equivocal for hypercortisolism, an ACTH stimulation test was performed. A population of randomly selected Miniature Schnauzers with normal fasting triglycerides underwent the same investigations. Based on the results of this testing, animals were classified as "healthy" (normotriglyceridaemia (NTG) with no concurrent disease), "HC-NTG" (hypercortisolism (HC) with NTG), "HC-HTG" (HC with HTG) or "primary HTG" (HTG with no secondary causes identified). From each dog enrolled in the study, once grouped to one of these categories, 10mls of blood

was collected into ethylenediaminetetraacetic acid (EDTA) for lipoprotein analysis. The sample was kept at 4°C before centrifugated at 1,358 g for 10 minutes at 4°C within 24 hours. The plasma was aliquoted and stored at -80°C until analysis. Blood collected for triglyceride and cholesterol measurement was placed into a plain tube following a 15-hour fast. The sample was centrifuged at 1,358 g for 10 minutes at 4°C before being aliquoted and frozen at -80°C until further processed.

Assays

Triglycerides and cholesterol were measured using enzymatic colorimetric assays on an automated spectrophotometer (COBAS INTEGRA® 400 plus analyzer, Roche Diagnostics, Basel, Switzerland).

Hematology was performed on either a Sysmex XN-1000V haematology analyser (Sysmex Corporation, Kobe, Japan) or ***** with a manual differential count included in each complete blood count. Biochemistry (including DGGR lipase) was performed using Roche reagents on the Cobas 8000 [c502 and c702 modules] (Roche Diagnostics, Indianapolis, IN, USA). Siemens Multistix 10 SG (Siemens Medical Solutions, Pennsylvania, USA) were used for urine dipstick analysis. A concurrent urine specific gravity and sediment examination was performed in all dogs. Total T4 and cTSH was performed on the Immulite 2000 (Immunoassay System (Siemens Medical Solutions Diagnostics, Flanders, NJ, USA). Cortisol concentrations were measured on the Advia Centaur XP Immunoassay System (Siemens Healthcare Diagnostics, Tarrytown, NY, USA).

Lipoprotein electrophoresis

Lipoprotein electrophoresis was performed on buffered (pH 8.5) agarose gel on a semi-automated instrument (Hydrasis, Sebia Italia S.r.l.), using a dedicated kit (Hydragel 15 lipoproteins) produced by the same manufacturer. Briefly, 10 µL of sample were loaded in each well of the applicator; then, the applicator was placed into a wet chamber to allow samples to diffuse onto the applicator teeth for 5 minutes. After the agarose gel and the sample applicators were loaded into the instrument, the migration at 160 V for 25 minutes was started. When the migration was completed, the gel was dried at 60°C for 15 minutes, stained with Sudan black, washed with ethanol (45%), dried and placed on the gel scanner. Gels were scanned at 570 nm and analyzed using the software Phoresis (Sebia Italia S.r.l., Bagno a Ripoli, Italy) that calculated the area under the peaks corresponding to HDL, VLDL and LDL.

Statistical analysis

Statistical analyses were performed using SPSS software (v.24, IBM) and a *p* value ≤ 0.05 was considered statistically significant. Data were tested for normality using Shapiro-Wilk test. Significant differences in lipid concentration between groups were assessed by Kruskal–Wallis test followed by Mann–Whitney U test.

Results

In total, 51 Miniature Schnauzers have been included in the study. Sixteen were healthy dogs, 9 were classified as HC-NTG, 19 classified as HC-HTG and 7 with primary HTG. The mean age was 8 years (range 9 months to 13 years). Twenty-seven (53%) dogs were female (1 intact and 26 neutered), and 24 dogs (47%) were male (2 intact and 22 neutered). The mean body condition score was 5 (range from 3

to 7 on a 9-point scale). Table 2 shows the age, gender and body condition score in each group. The only significant difference was a higher BCS in the primary HTG group compared with the healthy dog group (p=0.008). There were no significant differences for age and gender between the groups.

Triglycerides, cholesterol as well as lipoprotein electrophoretic fractions of the four groups of dogs (healthy, HC-NTG, HC-HTG and primary HTG) were compared. Triglyceride and cholesterol concentrations, LDL and HDL electrophoretic fractions displayed non-Gaussian distribution and non-parametric tests were used to compare the groups. Results are summarized in table 3.

Cholesterol and triglycerides (figures 1 and 2, respectively) were significantly higher in dogs with primary HTG (p=0.022 and p=0.000, respectively) and in dogs with HC-HTG (p=0.001 and p=0.000, respectively) than in healthy dogs. In dogs with HC-HTG both cholesterol and triglycerides were significantly higher (p = 0.037 and p=0.000, respectively) than in dogs with HC-NTG. Triglyceride concentration was significantly higher (p=0.000) in MS with primary HTG than in HC-NTG. The HDL percentage was significantly lower (p=0.028) in dogs with HC-HTG compared to HC-NTG (p=0.043) (figure 3). LDL percentage was significantly higher in MS with primary HTG than in HC-NTG (p=0.042) and in MS with HC-HTG than in HC-NTG (p=0.025). There were no significant differences in the VLDL and LDL percentages between the groups.

Discussion

As expected, hypertriglyceridemic dogs (primary HTG and HC-HTG) had significantly higher concentrations of triglycerides and cholesterol when compared to the normotriglyceridaemic dogs (healthy or HC-NTG). Unfortunately, dogs with primary HTG could not be distinguished from the other hypertriglyceridemic dogs based on the lipoprotein profile which did not show any significant difference.

Whilst the underlying pathological mechanism for primary HTG in the Miniature Schnauzer remains unknown, two studies have reported that dogs with primary HTG have increased VLDL concentrations, with or without an accompanying chylomicronaemia.[13,16] In some dogs with primary HTG, a concurrent decrease in LDL concentrations has also been detected.[13,16] In contrast to these previous publications, the results of this study detected increased LDL in primary HTG compared with HC-NTG, but no differences between MS with HC-HTG. No significant differences between VLDL or HDL concentrations between dogs with primary HTG and dogs in the other groups. One possible explanation could be the small sample size of dogs with primary hyperlipidemia, a type II error. Our study, which recruited 215 Australian Miniature Schnauzers only identified 7 dogs with primary HTG. As such, the disease, as a single entity is considered rare in Australia which made recruitment of a higher number of dogs difficult. Another possible explanation is that rigorous adrenal function testing was performed in the current study. Hypercortisolism has been reported to increase triglyceride, cholesterol and VLDLs concentrations[7]. Previous studies investigating hyperlipidemia in Miniature Schnauzers did not employ such rigorous testing to exclude HC as a cause of HTG before lipoprotein profiling, it is thus possible that the increased concentrations of VLDLs in these previous studies were secondary to undiagnosed HC.

In the current study, dogs were diagnosed with primary HTG after exclusion of all causes of secondary HTG including the comprehensive exclusion of HC based on the latest European Society of Veterinary Endocrinology ALIVE Definitions and American College of Veterinary Internal Medicine consensus statement[24]. In humans, certain diseases have lipoprotein profiles equivalent to a fingerprint. Given the similar lipoprotein profiles of primary HTG and HC, the lack of significant difference between the two diseases in this study, the recognition of sub-diagnostic HC in the ALIVE Definitions and the fact that primary HTG is a diagnosis of exclusion, HTG as a primary disease in Miniature Schnauzers requires further investigation. It is possible that Miniature Schnauzers previously diagnosed with primary HTG in previous studies were affected by HC, especially as the condition is recognized to increase in prevalence with ageing similar to HC. In the present study, multiple tests have been performed to identify sub-diagnostic HC. The relatively high number of MS with HTG that tested positive for HC suggests that a diagnosis of HC should be rigorously excluded before diagnosing primary HTG in MS; a diagnosis based on exclusion. Alternatively, it could be that Miniature Schnauzers, at least in Australia, have concurrent primary HTG and HC. The resolution of HTG with tight control of HC in a small number of dogs followed up from the study, suggests that this would not explain all such cases.

There are few studies investigating the lipoprotein profiles in dogs with HC. When lipoprotein profiles assessed by chromatographic analysis were compared between healthy dogs, obese dogs and those with HC, HC dogs were found to have higher total concentrations of triglycerides, cholesterol and VLDLs[7]. In addition, HDL-

cholesterol fractions were significantly lower in dogs with HC[7]. The results of the present study revealed a lower HDL percentage in dogs with HC-HTG compared to HC-NTG. The reason why there was no significance difference observed in the VLDL concentrations between the healthy group and those with HC remains unknown although it may be associated with the wide variation in VLDL percentages (table 3). Another possible explanation could be the different sensitivity across different analytical methods. Chromatographic analysis or separation per density gradient could have a higher sensitivity compared with semi-quantitative electrophoretic separation. Hypercholesterolemia is variably present in Miniature Schnauzers with HTG. Although Miniature Schnauzers in our study with HTG (primary HTG and HC-HTG) had significantly higher concentrations of cholesterol when compared to the normotriglyceridaemic dogs (healthy or HC-NTG), the absolute cholesterol concentrations were typically within laboratory reference intervals. This finding is similar to that reported previously[21].

A limitation of this study was the lack of a gold standard for the measurement of VLDL, LDL and HDL concentrations in canine plasma. The methodologies used to study canine lipoproteins include electrophoresis, sequential density gradient centrifugation, and size exclusion methods[1,8,9,16,25–29]. Sequential density gradient centrifugation is the gold standard in human medicine. In dogs, LDL and HDL span the density range of 1.006–1.087 g/dL and 1.025–1.21 g/dL, respectively with a significant overlap precluding complete separation by ultracentrifugation. This overlap is due to the presence of large, cholesterol enriched HDL particles, called HDL_1 in the density range 1.025-1.10 g/L. Canine HDL contains a second, relatively smaller and denser subpopulation of particles in the density range 1.07-1.21 g/L, which although similar to human HDL_3 are usually referred to as HDL_2[9]. These

lipoproteins are deficient in cholesterol and apoE relative to HDL_1 and appear to function as the efflux acceptors of free cholesterol from peripheral tissues in the initial stages of reverse cholesterol transport. Given the presence of HDL_1 in the LDL density range, ultracentrifugation is not the method of choice for dogs. Ultracentrifugation method is not available in Western Australia, posing a major technical barrier; thus, we opted for lipoprotein electrophoresis. Electrophoretic methods separate canine lipoproteins efficiently and are generally accepted as accurate, even if differences between automated and manual electrophoresis have been demonstrated, particularly with their ability to identify canine VLDL[30]. The differences in migration patterns may be partially attributed to the formulations of the gels or buffer used in different systems.

In conclusion, it is not possible to clearly identify a specific lipoprotein pattern in dogs with primary HTG or with hypercortisolism using lipoprotein electrophoresis method. Therefore, the diagnosis of primary HTG diagnosis still remains a diagnosis of exclusion requiring extensive endocrine testing in addition to routine hematology and serum biochemistry. Lipoprotein profiling using a more sensitive method, as proposed by Xenoulis and colleagues may be warranted in a similar population of MS.

References

1. Rogers WA, Donovan EF, Kociba GJ. Lipids and lipoproteins in normal dogs and in dogs with secondary hyperlipoproteinemia. *J Am Vet Med Assoc*. 1975;166(11):1092-1100.

2. Ginsberg HN. Lipoprotein physiology. *Endocrinology and metabolism clinics of North America*. 1998;27(3):503-519.

3. Xenoulis PG, Steiner JM. Lipid metabolism and hyperlipidemia in dogs. *The Veterinary Journal*. 2010;183(1):12-21.

4. Watson T, Practice JBJ of SA. Lipoprotein metabolism and hyperlipidaemia in the clog and cat: A review. *Journal of Small Animal Practice*. 1993;34(10):479-487.

5. Wilkins JT, Li RC, Sniderman A, Chan C, Lloyd-Jones DM. Discordance Between Apolipoprotein B and LDL-Cholesterol in Young Adults Predicts Coronary Artery Calcification The CARDIA Study. *J Am Coll Cardiol*. 2016;67(2):193-201.

6. Tani S, Yagi T, Atsumi W, Kawauchi K, Matsuo R, Hirayama A. Relation between low-density lipoprotein cholesterol/apolipoprotein B ratio and triglyceride-rich lipoproteins in patients with coronary artery disease and type 2 diabetes mellitus: a cross-sectional study. *Cardiovasc Diabetol*. 2017;16(1):123.

7. Jericó MM, Chiquito FDC, Kajihara K, et al. Chromatographic analysis of lipid fractions in healthy dogs and dogs with obesity or hyperadrenocorticism. *Journal of Veterinary Diagnostic Investigation*. 2009;21(2):203-207.

8. Solyom A, Bradford RH, Furman RH. Apolipoprotein and lipid distribution in canine serum lipoproteins. *Biochimica et biophysica acta*. 1971;229(2):471-483.

9. Mahley RW, Weisgraber KH. Canine lipoproteins and atherosclerosis. I. Isolation and characterization of plasma lipoproteins from control dogs. *Circulation research*. 1974;35(5):713-721.

10. Oda H, Mori A, Hirowatari Y, et al. Cholesterol concentrations in lipoprotein fractions separated by anion-exchange–high-performance liquid chromatography in healthy dogs and dogs with hypercholesterolemia. *Research in Veterinary Science*. 2017;114:163-169.

11. Suto A, Yamasaki M, Takasaki Y, et al. LC-MS/MS Analysis of Canine Lipoproteins Fractionated Using the Ultracentrifugation-Precipitation Method. *J Vet Medical Sci*. 2013;75(11):1471-1477.

12. Pischon T, Girman CJ, Sacks FM, Rifai N, Stampfer MJ, Rimm EB. Non–High-Density Lipoprotein Cholesterol and Apolipoprotein B in the Prediction of Coronary Heart Disease in Men. *Circulation*. 2005;112(22):3375-3383.

13. Xenoulis PG, Cammarata PJ, Walzem RL, Macfarlane RD, Suchodolski JS, Steiner JM. Novel lipoprotein density profiling in healthy dogs of various breeds, healthy Miniature Schnauzers, and Miniature Schnauzers with hyperlipidemia. *BMC Veterinary Research*. 2013;9(1):47.

14. Barrie J, Watson T. Plasma cholesterol and lipoprotein concentrations in the dog: The effects of age, breed, gender and endocrine disease. *Journal of Small Animal Practice*. 1993;34(10):507-512.

15. Downs LG, Bolton CH, Crispin SM, Wills JM. Plasma lipoprotein lipids in five different breeds of dogs. *Research in Veterinary Science*. 1993;54(1):63-67.

16. Whitney MS, Boon GD, Rebar AH, Story JA, Bottoms GD. Ultracentrifugal and electrophoretic characteristics of the plasma lipoproteins of miniature schnauzer dogs with idiopathic hyperlipoproteinemia. *Journal of veterinary internal medicine*. 1993;7(4):253-260.

17. Seage EC, Drobatz KJ, Hess RS. Spectrophotometry and Ultracentrifugation for Measurement of Plasma Lipids in Dogs with Diabetes Mellitus. *J Vet Intern Med*. 2018;32(1):93-98.

18. Furrow E, Jaeger JQ, Parker VJ, et al. Proteinuria and lipoprotein lipase activity in Miniature Schnauzer dogs with and without hypertriglyceridemia. *The Veterinary Journal*. 2016;212(C):83-89.

19. Lewis GF, Xiao C, Hegele RA. Hypertriglyceridemia in the Genomic Era: A New Paradigm. *Endocr Rev*. 2015;36(1):131-147.

20. Xenoulis PG, Tate NM, Bishop MA, Steiner JM, Suchodolski JS, Furrow E. Sequence analysis of the coding regions of the apolipoprotein C2 (APOC2) gene in Miniature Schnauzers with idiopathic hypertriglyceridemia. *Vet J*. 2020;265:105559.

21. Xenoulis PG, Suchodolski JS, Levinski MD, Steiner JM. Investigation of hypertriglyceridemia in healthy Miniature Schnauzers. *Journal of veterinary internal medicine*. 2007;21(6):1224-1230.

22. Xenoulis PG, Cammarata PJ, Walzem RL, Suchodolski JS, Steiner JM. Effect of a low-fat diet on serum triglyceride and cholesterol concentrations and lipoprotein profiles in Miniature Schnauzers with hypertriglyceridemia. *J Vet Intern Med*. 2020;34(6):2605-2616.

23. Imbery CA, Dieterle F, Ottka C, et al. Metabolomic Abnormalities in Serum from Untreated and Treated Dogs with Hyper- and Hypoadrenocorticism. *Metabolites*. 2022;12(4):339.

24. Behrend EN, Kooistra HS, Nelson R, Reusch CE, Scott-Moncrieff JC. Diagnosis of Spontaneous Canine Hyperadrenocorticism: 2012 ACVIM Consensus Statement (Small Animal). *J Vet Intern Med*. 2013;27(6):1292-1304.

25. Pasquini A, Luchetti E, Cardini G. Plasma lipoprotein concentrations in the dog: the effects of gender, age, breed and diet. *J Anim Physiol An N*. 2008;92(6):718-722.

26. Whitney MS, Boon GD, Rebar AH, Ford RB. Effects of acute pancreatitis on circulating lipids in dogs. *Am J Vet Res*. 1987;48(10):1492-1497.

27. Chikamune T, Katamoto H, Ohashi F, Shimada Y. Serum lipid and lipoprotein concentrations in obese dogs. *Journal of Veterinary Medical Science*. 1995;57(4):595-598.

28. Chikamune T, Katamoto H, Nomura K, Ohashi F. Lipoprotein Profile in Canine Pancreatitis Induced with Oleic Acid. *J Vet Med Sci*. 1998;60(4):413-421.

29. Jeusette IC, Lhoest ET, Istasse LP, Diez MO. Influence of obesity on plasma lipid and lipoprotein concentrations in dogs. *American journal of veterinary research*. 2005;66(1):81-86.

30. Behling-Kelly E. Comparison of 2 electrophoretic methods and a wet-chemistry method in the analysis of canine lipoproteins. *Vet Clin Path*. 2016;45(1):124-134.

Tables:

Table 1: Summary of the biochemical markers included as a part of the biochemistry profile and the test principle for each biomarker

Biomarker	Test principle
Albumin	Colorimetric assay
Alkaline phosphatase	Colorimetric assay
Alanine aminotransferase	UV kinetic methodology without pyridoxal phosphate activation
Aspartate aminotransferase	UV kinetic methodology without pyridoxal phosphate activation
Bilirubin	Colorimetric diazo method
Gamma-glutamyl transferase	Enzymatic colorimetric assay
Sodium	Ion selective electrodes
Chloride	Ion selective electrodes
Potassium	Ion selective electrodes
Total calcium	Calcium ions react with 5-nitro-5'-methyl-BAPTA (NM-BAPTA) under alkaline conditions to form a complex. This complex reacts in the second step with EDTA
Cholesterol	Enzymatic colorimetric assay
Triglycerides	Enzymatic colorimetric assay
Amylase	Enzymatic colorimetric assay
Lipase	Enzymatic colorimetric assay with 1,2-O-dilauryl-rac-glycero-3-glutaric-acid- (6-methylresorufin) ester as substrate

Creatinine	Colorimetric assay based on Jaffé method
Urea	Kinetic test with urease and glutamate dehydrogenase
Phosphate	Molybdate UV
Bicarbonate	Enzymatic colorimetric assay using phosphoenolpyruvate (PEP)
Glucose	UV test using hexokinase
Total protein	Colorimetric assay using divalent copper
Creatinine kinase	UV test

Table 2: Age, body condition score (BCS) and gender distribution across different groups. FN: female neutered, MN: male neutered, F female, M: male.

	Age median (min-max)	BCS median (min-max)	Gender
Healthy	8 (9 months-12)	5 (4-6)	FN=10 MN=6
Primary hypertriglyceridemia	10 (4-13)	6 (5-7)	FN=3 MN=4
Hypercortisolism with hypertriglyceridemia	9 (2-13)	5 (3-7)	FN=11 MN=5 M=2 F=1
Hypercortisolism with normotriglyceridemia	8 (2-12)	5 (4-7)	FN=2 MN=7

Table 3: Descriptive data reporting the median and minimum-maximum for each measured variable for each group. HC-NTG = hypercortisolism with normotriglyceridemia; HC-HTG = hypercortisolism with hypertriglyceridemia; VLDL-EL = very-low-density lipoprotein when measured by electrophoresis; LDL-EL = low-density lipoprotein when measured by electrophoresis; HDL-EL = high-density lipoprotein when measured by electrophoresis

	Groups			
	Healthy	**Primary**	**HC-NTG**	**HC- HTG**
	Median (*min-max*)	Median (*min-max*)	Median (*min-max*)	Median (*min-max*)
Cholesterol mmol/L	4.57 (2.76-9.70)	6.55 (5.16-7.71)	5.70 (4.15-6.07)	7.32 (4.81-13.20)
Triglycerides mmol/L	0.95 (0.28-1.49)	3.76 (1.84-25.79)	0.91 (0.53-1.62)	6.57 (1.70-27.45)
VLDL-EL %	5.8 (1.10-34.40)	6.80 (4.20-14.10)	9.10 (2.60-11.10)	9.60 (1-72.70)
LDL-EL %	7 (2.60-20.70)	7.70 (4.10-30.40)	6.40 (2.10-11.40)	7.40 (3-23.4)
HDL-EL %	85.9 (60.90-94.20)	84 (61.20-91)	85.30 (79.50-90.10)	78.50 (23.3-91)

Figure 1: Box and whisker plots showing the cholesterol concentrations when measured with the biochemical assay between the different groups. The ends of the box are the upper and the lower quartiles; the box spans the interquartile range, and the central line is the median. The whiskers are the 2 lines outside the box that extend to the highest and lowest observation. NTG = normotriglyceridemia; HTG =

hypertriglyceridemia; HC-HTG = hypercortisolism with hypertriglyceridemia; HC-NTG

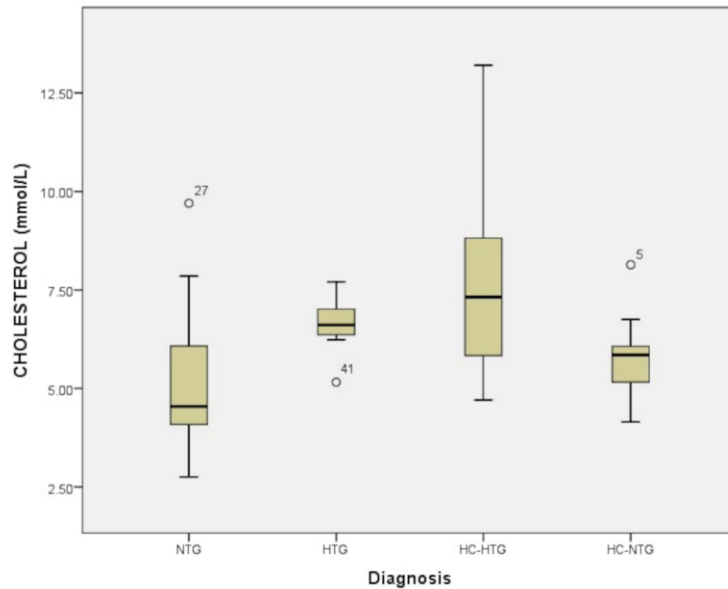= hypercortisolism with normotriglyceridemia

Figure 2: Box and whisker plots showing the triglycerides concentrations when

measured with the biochemical assay between the different groups. The ends of the

box are the upper and the lower quartiles; the box spans the interquartile range, and

the central line is the median. The whiskers are the 2 lines outside the box that

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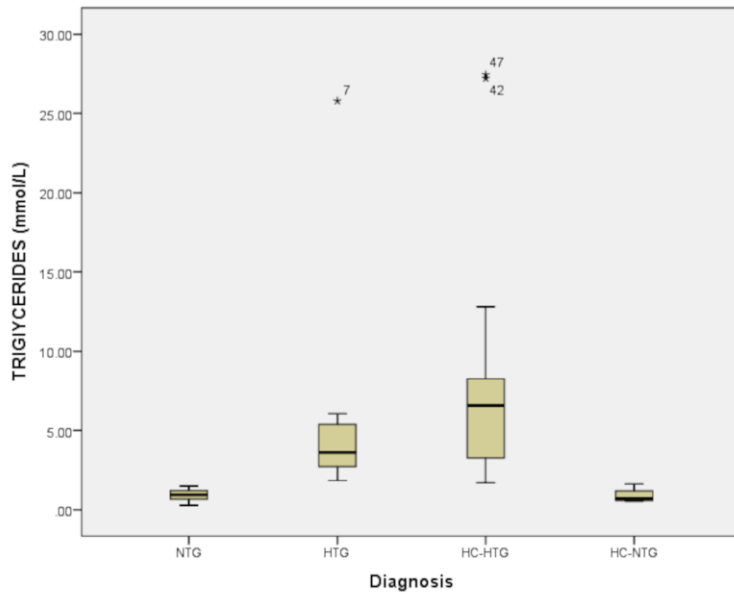
= hypercortisolism with normotriglyceridemia

Figure 3: Box and whisker plots showing the HDL percentage when measured with the electrophoresis between the different groups. The ends of the box are the upper and the lower quartiles; the box spans the interquartile range, and the central line is the median. The whiskers are the 2 lines outside the box that extend to the highest and lowest observation. NTG = normotriglyceridemia; HTG = hypertriglyceridemia; HC-HTG = hypercortisolism with hypertriglyceridemia; HC-NTG = hypercortisolism with normotriglyceridemia