Body condition score and triglyceride concentrations and their associations with other markers of energy homeostasis in healthy, non-obese dogs

Carlos Gomez-Fernandez-Blanco[1*], Dominique Peeters[1], Frédéric Farnir[2], Katja Höglund[3], Vassiliki Gouni[4,5], Maria Wiberg[6], Jakob Lundgren Willesen[7], Sofia Hanås[3,8], Kathleen McEntee[1,9], Laurent Tiret[4,5], Jens Häggström[3], Hannes Lohi[10], Valérie Chetboul[4,5], Merete Fredholm[7], Eija Seppälä[10], Anne-Sophie Lequarré[1], Alexander James German[11], Anne-Christine Merveille[1]

[1]Department of clinical sciences, College of Veterinary Medicine, University of Liège, Liège, Belgium.

[2]Department of sustainable animal resources, FARAH, College of Veterinary Medicine, University of Liège, Liège, Belgium.

[3]Swedish University of Agricultural Sciences, Uppsala, Sweden.

[4]Université Paris-Est Créteil, INSERM, IMRB, Créteil, France.

[5]École nationale vétérinaire d'Alfort, IMRB, Maisons-Alfort, France.

[6]Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Finland.

[7]Department of Veterinary and Animal Sciences, University of Copenhagen, Copenhagen, Denmark.

[8]Evidensia Specialist Animal Hospital Strömsholm, Strömsholm, Sweden.

[9]Faculty of Medicine, Université Libre de Bruxelles (ULB), Brussels, Belgium.

[10]Department Medical and Clinical Genetics, and Department of Veterinary Biosciences, University of Helsinki, Finland.

[11]Institute of Life Course and Medical Sciences, University of Liverpool, Liverpool, United Kingdom.

*Corresponding author:

cgfernandezblanco@gmail.com

Abstract

Serum triglyceride concentrations increase in dogs in overweight condition, which is typically assessed by body condition score (BCS). However, their associations with other markers of energy homeostasis are poorly characterized. The present study aimed to evaluate the associations between both BCS and triglyceride levels and other markers of lipid and glucose metabolism in healthy dogs in overweight condition. 534 overweight, but otherwise healthy, client-owned dogs were included. Serum concentrations of cholesterol, free fatty acids, triglycerides, insulin, glucose and fructosamine were measured. Dogs were assigned to lean (BCS: 3-5) or overweight (BCS: 6-7) categories, and linear models were used to assess the differences between BCS categories and the associations between triglycerides and the other variables, correcting for the effect of breed. Globally, “overweight” dogs had greater serum cholesterol (95% CI: 5.3-6.2 mmol/L or 205-237 mg/dL versus 5.1-5.4 mmol/L or 198-210

mg/dl, P = .003), insulin (95% CI: 17.5-22.1 µU/ml versus 16.7-18.0 µU/ml, P = .036) and were older (95% CI: 4.0-5.3 versus 3.4-3.7 years, P = .002) than lean dogs. Triglyceride concentrations were positively associated with fructosamine (r2 = 0.31, P = .001), cholesterol (r2 = 0.25, P < .001), insulin (r2 = 0.14, P = .003) and glucose (r2 = 0.10, P = .002), and negatively associated with free fatty acids (r2 = 0.11, P < .001). There was no association between triglyceride levels and age. In conclusion, both BCS and triglyceride concentrations were associated with other markers of glucose and lipid metabolism in overweight, but otherwise healthy dogs. Triglyceride concentrations were associated with an increase in insulin and fructosamine that might reflect an early-phase impairment in glucose tolerance which, surprisingly, was concurrent with lower basal free fatty acids.

Introduction

Metabolic syndrome (MS) is an entity comprising multiple cardiovascular and metabolic risk factors in humans, characterized by a state of chronic, subclinical inflammation (1). Features of human MS include combinations of increased visceral fat (abdominal obesity), systemic hypertension, increased circulating triglyceride (TG) concentrations, decreased high-density lipoprotein (HDL) cholesterol concentration, and increased fasting glucose concentration (suggesting insulin resistance) (2). Human beings with MS have a greater risk of developing type 2 diabetes mellitus (DM) and cardiovascular complications (2).

Dogs also suffer from obesity-related metabolic dysfunction (ORMD), but the term metabolic syndrome is avoided because, even though dogs share some of its components (such as hypercholesterolaemia, hypertriglyceridaemia and insulin resistance), they do not develop the obesity-related diseases that humans do, such as atherosclerosis, stroke or type 2 DM (3–6). Studies on canine ORMD have mostly examined dogs with manifest obesity, whilst metabolic dysfunction of dogs in overweight condition has been less well characterized, despite its known associated with comorbidities in the canine species (7–10)**.**

Some people with obesity remain metabolically healthy, at least in the short term (11), during which time their risk of comorbidities is less, they do not develop insulin resistance, are normotensive, and concentration of glucose, TG, HDL cholesterol and high-sensitivity C-reactive protein (hsCRP) is within reference limits (12). Conversely, some normal-weight individuals develop the characteristics of MS despite having a normal body fat mass (13). Such 'metabolically unhealthy normal-weight' people can be identified by a thorough biochemical assessment in which increased TG and C-reactive protein (CRP) concentration, as well as decreased HDL-cholesterol and adiponectin concentrations will be found (13). Similarly, different biochemical phenotypes have been described in obese dogs: adiponectin was lesser and plasma insulin was greater in obese dogs that met the criteria of ORMD (which were defined as having obesity plus two other criteria amongst TG > 200 mg/dL (2.26 mmol/L), total cholesterol > 300mg/dL (7.8 mmol/L), systolic blood pressure >160 mmHg, and fasting plasma

glucose > 100mg/dL (5.6 mmol/L), or previously diagnosed diabetes mellitus) as compared to obese dogs without ORMD (14,15).

The aim of this study was to investigate associations amongst body condition score (BCS) and a range of metabolic variables associated with glucose and fat metabolism in a large cohort of healthy dogs in overweight condition, as compared to lean dogs, as well as to assess whether TG concentrations can be a marker for these metabolic variables.

Materials and methods

A canine database aimed at examining genetic determinants of disease (European LUPA project (16)) was retrieved. Five centres had participated in the original study between 2009 and 2010: University of Liège, University of Copenhagen, Swedish University of Agricultural Sciewnces, University of Helsinki, and the National Veterinary School of Maisons-Alfort (France). All centres used the same standardised protocols for recruitment and characterisation of dogs. The database was then retrospectively investigated for the purpose of the present work.

Dogs

This study was approved by the Ethical Committee of the LUPA project, and the informed consent of all owners was obtained (16). Client-owned, pure-bred dogs were recruited, and included different breed cohorts in order to represent a wide range of various phenotypic features. Dogs were 1 to 7 years old and were genetically unrelated. In order to minimise potential effects

of the oestrous cycle on metabolic parameters, each breed cohort comprised a single sex, namely intact males or female dogs that were spayed or in anoestrus (checked by a vaginal smear). Health status was checked through history, physical examination, laboratory analyses (including routine hematology and serum biochemistry), and a thorough cardiovascular investigation comprising ECG recording and echocardiographic examination. After visual assessment and palpation, each dog was assigned a BCS category using the one-to-nine point scale (17); dogs were then assigned to one of two body condition categories (lean, BCS 3-5; overweight, BCS 6-7) based on this score. Dogs were excluded if they showed clinical signs of any disease, were very underweight (BCS ≤2) or had obesity (BCS 8-9, as defined by Brooks et al. (18)).

Sampling

Three weeks before the study, owners were asked to feed their dogs exclusively with a commercial dry food diet of their choice, avoiding any treats or table food. Dogs were fasted for at least 12 hours before blood sampling and, after collection, blood was centrifuged within 30 minutes and serum then aliquoted. In most centres, serum aliquots were immediately frozen at -80°C; in the remaining centre, samples were frozen at -20°C for the first 2 weeks after collection, before being transferred to a -80°C thereafter. All samples were subsequently sent to the same laboratory[a] for analysis.

Analyses

The analytes selected for the present study were chosen based on their reported association with obesity in humans and dogs (2,3,14,19–41). These included markers of lipid metabolism (e.g., cholesterol, TG and free fatty acids, FFA), glucose homeostasis (e.g., glucose, fructosamine, and insulin) and inflammation (CRP). A photometric clinical chemistry analyzer (Konelab 60i, Thermo Electron Co, Finland) was used to determine fructosamine, glucose, CRP, cholesterol and TG concentrations. Fructosamine and FFA concentrations were respectively assessed using Hariba ABX (Montpellier, France) and Wako Chemical Gmbh (Neuss, Germany). Insulin concentration was determined by radioimmunoassay, provided by DiaSorin S.p.A (Italy).

Repeatability of measurements

Ten dogs were randomly selected to send duplicates to the same laboratory three months after the first analyses. Coefficients of variation were 6% for insulin and ≤5% for FFA, cholesterol, TG, glucose and fructosamine.

Statistical analyses

Outliers were inspected with the Reference Value Advisor (42) using the Tukey method (43). Dogs with only one outlier were accepted and included in the analyses; those with more than one outlying value were excluded.

Preliminary tests

Given that parametric tests were needed to test our hypotheses, all analyses were validated after checking the normal distribution of the residuals (evaluated by histogram observation and by a Shapiro-Wilk test), and a test of homoscedaticity (Breusch-Pagan test). If a particular analyte (dependent variable) did not pass the normality tests, a non-parametric equivalent test was used (e.g., Spearman's Rank correlation or Mann-Whitney test). Statistical tests were performed with R free statistical software (44).

Effect of age on biochemical variables

The correlation between age and each of the analytes was calculated by Pearson's method, and *P*-values <0.05 were considered to be statistically significant.

Effect of overweight status

In order to test for differences between lean and overweight groups, a linear model including BCS, breed and an interaction between BCS and breed was used for all biochemical variables. Normality was checked using the Shapiro-Wilk test and analytes with non-normal residuals were log-transformed. Type III sums of squares were used and differences with *P*-values <0.05 were considered to be statistically significant. Whenever a statistically significant interaction was found, associations within each breed were examined. In this case, a Mann-Whitney test was used and the level of significance was corrected for multiple comparisons using a Bonferroni correction.

Effect of TG on the biochemical variables

Linear models including TG, the breed and an interaction between the breed and TG were used for each biochemical variable. Once again, *P*-values <0.05 were considered to be statistically significant. Whenever an interaction with the breed was significant, the association between TG and the outcome variable was tested within each breed, using Spearman's correlation method. Once again, a Bonferroni correction was used to correct for multiple comparisons.

Results

Animals

In total, 534 dogs met the inclusion criteria, with 9 different breeds represented: Boxer (BOX, 15 dogs), Belgian Shepherd Dog (BSD, 125 dogs), Cavalier King Charles Spaniel (CKCS, 35 dogs), Dachshund (DACH, 40 dogs), Doberman (DOB, 39 dogs), Finnish Lapphund (FL, 45 dogs), German Shepherd dog (GSD, 66 dogs), Labrador Retriever (LAB, 125 dogs), and Newfoundland (NF, 44 dogs). All cohorts comprised only male dogs, except for the NF cohort, that comprised only females, and the LAB cohort, that comprised dogs of both sexes (73 females and 52 males). Some breeds were unique to one centre while others were shared among centres. Distribution of dogs by centre, breed, and gender is shown in Table 1. All outliers were used in the statistical analyses, since they were considered compatible with physiological values, and no dog showed more than one outlier result.

166 **Table 1**. Distribution of dogs by centre, breed, and sex

	Belgium	Denmark	Finland	France	Sweden	Total
BSD	**97M**			**28M**		**125**
BOX					**15M**	**15**
CKCS					**35M**	**35**
DACH			**24M**		**16M**	**40**
DOB				**39M**		**39**
FL			**45M**			**45**
GSD	**17M**		**49M**			**66**
LAB	**7M**	**44F**		**29F**	**45M**	**125**
NF		**44F**				**44**
Total	**121**	**88**	**118**	**96**	**111**	**534**

167 M, Male ; F, Female ; BOX, Boxer; BSD, Belgian Sheperd Dog; CKCS, Cavalier King Charles

168 Spaniel; DACH, Dachshund; DOB, Doberman pinscher; FL, Finnish Lapphund; GSD, German

169 Shepherd Dog; LAB, Labrador retriever; NF, Newfoundland.

Median BCS was 3 in FL (interquartile range, IQR: 3-4); 4 in GSD (IQR: 3-4), DACH (IQR: 3-5), BSD (IQR: 4-5) and BOX (IQR: 4-5); 5 in DOB (IQR: 5-5), CKCS (IQR: 5-6) and NF (IQR: 5-6); and 5.5 in LAB (IQR: 5-6). Seventy-one percent of the dogs (409) were in the lean category, while 29% (120 dogs) were overweight. Table 2 shows the distribution of BCS amongst breeds.

Table 2. Distribution of dogs by breed and BCS.

	BCS 2	**BCS 3**	**BCS 4**	**BCS 5**	**BCS 6**	**BCS 7**
BOX	-	-	8	4	3	-
BSD	1	22	45	51	5	1
CKCS	-	-	-	20	15	-
DACH	-	11	12	11	6	-
DOB	-	-	2	33	4	-
FL	-	24	12	6	2	1
GS	1	22	35	4	1	-

LAB	-	1	8	53	59	4
NF	-	-	-	23	15	4
TOTAL	**2**	**80**	**122**	**205**	**110**	**10**

BCS, Body Condition Score ; BOX, Boxer; BSD, Belgian Sheperd Dog; CKCS, Cavalier King Charles Spaniel; DACH, Dachshund; DOB, Doberman pinscher; FL, Finnish Lapphund; GSD, German Shepherd Dog; LAB, Labrador retriever; NF, Newfoundland.

Analyses were validated after successfully testing for normal distribution of the residuals and homoscedaticity. CRP was the only variable that did not succeed the tests and was, therefore, assessed with nonparametric methods.

Association between age and biochemical variables

There was no effect of age on any analyte tested (cholesterol, P=0.49; FFA, P=0.88; TG, P=0.55; CRP, P=0.34; insulin, P=0.18; glucose, P=0.65; fructosamine, P=0.11).

Effect of overweight status on biochemical variables

After normalisation for the effect of the breed, dogs in the overweight category were older (P=0.002) and had greater plasma insulin (P=0.036) and cholesterol (P=0.003) concentrations than lean dogs. An interaction between BCS category and breed was also identified for both

TG and cholesterol (Table 3): in this respect, cholesterol concentration was greater in overweight BOX (*P*=0.020) and CKCS (*P*=.0005) compared with their lean counterparts; overweight CKCS also had greater TG than lean CKCS (*P*=0.002). These differences are illustrated in Fig 1.

Table 3. Results of ANOVA. Effect of BCS category, and its interaction with the effect of the breed, on age (years) and on concentrations of insulin, fructosamine, glucose, cholesterol, free fatty acids, and triglycerides in overweight and lean dogs. Data are presented as mean and 95% confidence intervals.

	Lean	**Overweight**	**BCS category,** p-value	**Interaction between BCS category and breed,** p- value
Age (years)	3.5 (3.4-3.7)	4.4 (3.9-4.9)	0.**0005**[a]	0.3405
Insulin (pmol/l)	120.1 (113.2-125.7)	131.3 (122.2-141.7)	0.**0374**[a]	0.1289

Insulin (µU/ml)	17.3 (16.7-18.1)	18.9 (17.6-20.4)		
Fructosamine (µmol/l)	287.1 (283.6 – 290.6)	289.7 (283.2 – 296.2)	0.4951	0.4560
Glucose (mmol/l)	0.05 (0.05-0.05)	0.05 (0.05-0.06)	0.3828	0.6783
Glucose (mg/dl)	0.98 (0.97-0.99)	0.99 (0.97-1.01)		
Cholesterol (mmol/l)	5.3 (5.1-5.4)	5.7 (5.3-6.2)	0.**0032**[a]	0.**0006**[b]
Cholesterol (mg/dl)	204 (198-210)	221 (205-237)		
FFA (mEq/l)	0.87 (0.83-0.91)	0.86 (0.78-0.93)	0.7657	0.7529

Triglycerides (mmol/l)	0.005 (0.005-0.005)	0.005 (0.005-0.006)	0.9716	0.**0019**[b]
Triglycerides (mg/dl)	0.43 (0.42-0.45)	0.45 (0.40-0.51)		

197 FFA, free fatty acids.

198 [a] Significant effect of the BCS category for a level of confidence of 0.05.

199 [b] Significant interaction between breed and BCS category, for a level of confidence of 0.05.

200 **Fig. 1.** Box plots showing serum cholesterol (A and B) and triglyceride (C) concentrations in
201 Boxer (BOX) and Cavalier King Charles Spaniel (CKCS). Bonferroni-corrected p-value of
202 0.0056. The lower, middle and upper line of each box represent the 25th percentile (bottom
203 quartile), 50th percentile (median) and the 75th percentile (top quartile). The whiskers, where
204 present, represent the minimum and maximum. Outliers, represented by open circles, were
205 included in the analyses.

206 Associations between TG concentration and markers of ORMD

207 After normalisation for the effect of the breed, TG concentrations were positively associated
208 with fructosamine (P=0.001), cholesterol (P<0.001), insulin (P=0.003) and glucose (P=0.001)
209 concentrations, and negatively associated with FFA (P<.0001). Results are shown in **Table 4**.

The interaction between TG and breed was significant when testing insulin concentration as the dependent variable and, in the intra-breed analyses, there was a positive association between TG and insulin concentrations in several breeds (**Table 5**).

Table 4. Association between triglyceride concentration and insulin, fructosamine, glucose, cholesterol, and free fatty acids. The r^2 corresponds to the predictive percentage attributed to the whole linear model, which included the main effects of both triglycerides and breed (plus their interaction, when significant) as explanatory variables.

	Adjusted r2	Triglyceridaemia, p-value	Interaction between triglyceridaemia and breed, p-value
Insulin	0.16	**0.0030**[a]	**0.0101**[b]
Fructosamine	0.31	**0.0013**[a]	0.0711
Glucose	0.10	**0.0014**[a]	0.7750
Cholesterol	0.25	**<0.0001**[a]	0.1572
FFA	0.10	**<0.0001**[a]	0.0645

FFA, free fatty acids.

[a] Significant effect of triglyceridaemia, for a level of confidence of 0.05.

[b] Significant interaction between breed and triglyceride concentrations, for a level of confidence of 0.05.

Table 5. Correlations between triglyceride concentration (mg/dl) and insulin (µU/ml) within individual breeds. Spearman's rank correlation test.

Breed	p-value	Spearman's R
BOX	0.0066	0.67
BSD	0.0008	0.30
CKCS	0.5361	0.11
DACH	0.0819	0.28
DOB	0.0515	0.39
FL	0.6246	-0.07
GS	0.1973	0.16
LAB	0.0305	0.20
NF	0.1018	0.26

223 BOX, Boxer; BSD, Belgian Sheperd Dog; CKCS, Cavalier King Charles Spaniel; DACH,

224 Dachshund; DOB, Doberman pinscher; FL, Finnish Lapphund; GSD, German Shepherd Dog;

225 LAB, Labrador retriever; NF, Newfoundland.

226

Discussion

Two main findings can be highlighted from this large cohort of dogs recruited from various European countries. First, insulin and cholesterol concentrations are increased in dogs in overweight body condition; second, fasting TG concentrations are positively associated with cholesterol, glucose, fructosamine and insulin, but negatively associated with FFA. Previous studies, often experimental, have reported increased insulin, cholesterol and TG concentrations in obese dogs. However, the "overweight" group in those studies invariably comprises either some or all dogs in obese body condition (BCS 8-9) (14,19,23,35–38,45–47). To the best of our knowledge, the present study is the first to report similar changes in a large group of dogs in overweight condition only (BCS 6-7). Currently, consensus does not exist on when accumulated adipose tissue becomes pathologicial in dogs and, in some previous studies, dogs with BCS 6/9 have been included in the "ideal weight" group, together with BCS 4 and BCS 5 (32,48,49). Conversely, some studies have examined "mildly to moderately overweight" dogs separately from "obese" dogs and found both groups to be at greater risk for developing comorbidities, suffering from a poorer quality of life and having a shorter life-expectancy (7–10). For example, Kealy et al. (2002) found that long-term food-restricted Labrador Retriever dogs had a longer life span and delayed onset of chronic diseases as compared to a control group (8). There was a mean difference of 26% between groups, which was reflected by a difference in BCS (mean BCS 4.6 +/- 0.19 in the food-restricted group, versus BCS 6.7 +/- 0.19 in the control group). In the present study, insulin and cholesterol concentrations were significantly

247 greater in a cohort of overweight dogs (median = 6; IQR = 0; mean BCS = 6.1; range, 6 – 7)
248 than in their lean counterparts (median BCS = 5; IQR =1; mean BCS = 4.3; range, 2 – 5).

249 In addition to the main effects, both TG and cholesterol concentrations were affected by an
250 interaction between overweight status and breed. When such an interaction between two
251 independent variables is found, interpretation of the main effects alone may be misleading and,
252 therefore, each category (in this case, individual breeds) should be investigated independently
253 (50). In this respect, compared with lean status, overweight status was associated with greater
254 TG concentrations in the CKCS, and also greater cholesterol concentrations in both BOX and
255 CKCS breeds. We hypothesize that the positive results within these breeds might be related to
256 the experimental design of the present study, rather than a breed-specific cause.
257 Hyperinsulinaemia is thought to be key in obesity-related disorders in humans (51). Even
258 though hyperinsulinaemia is also a feature of canine obesity, dogs do not develop the same
259 outcomes as humans with MS (14), suggesting that significant physiological and
260 pathophysiological differences might exist between these species (3). In the current study,
261 overweight status in dogs was associated with increased concentrations of insulin and
262 cholesterol, which might be interpreted as an early evidence of ORMD. These changes,
263 although mild and likely not clinically relevant, might contribute to the long-term consequences
264 of fat accumulation (i.e., reduced lifespan and quality of life, rapid onset of comorbidities) that
265 have been described in overweight dogs (7–10).

In contrast to TG and cholesterol, FFA concentrations were not different between dogs in the overweight and lean categories. Previous studies have shown that both humans and dogs with obesity have increased FFA concentrations, possibly because their concurrent insulin resistance leads to a lack of insulin-mediated suppression of lipolysis (36,37,46,51); further, FFA are considered to be key mediators in the pathogenesis of obesity-induced insulin resistance (51). Therefore, the degree of adiposity in the dogs of the current study might have been less than that required to affect circulating FFA concentrations.

Fasting hyperglycaemia is seen in humans with obesity (52), and is a risk factor for progression to type 2 DM (2). In dogs, type 2 DM has not been convincingly described (53,54), but the incidence of diabetes mellitus has increased in the last years, paralleling the increase in obesity (8,55,56), whilst several studies report obesity to be a risk factor (57,58).

In the current study, there was no difference in glucose concentrations between the dogs in the overweight and lean categories. Several canine studies have identified changes in glucose concentration associated with overweight status, weight gain and weight loss (39–41,59), but no such association is evident in other studies (14,19–21,24,35,37,60,61). In the present study, fructosamine concentration was did not differ between the dogs in the overweight and lean categories.

Fructosamine is not often included in studies on canine obesity. One study found greater fructosamine concentrations in insulin-resistant, but not insulin-sensitive, dogs with obesity

(27). The influence of obesity on fructosamine concentrations in humans is believed to be mild (28,29).

Some of the breeds included in the current study were overrepresented in the overweight category. This finding was expected for both LAB (62,63) and NF (18), breeds that have previously been reported to be predisposed to obesity (18,30,62,63). In contrast, some other obesity-prone breeds were under-represented in the overweight category, for example, there were only 15% and 20% of overweight dogs in the DACH and BOX categories, respectively (30).

There was no difference in CRP concentration between dogs in the overweight and lean categories. CRP is a marker of inflammation and is increased in humans with obesity (31). Subclinical inflammation is also a feature of obesity in dogs, and some authors have found a positive association between CRP and either obesity or weight loss in dogs (20,32). However, this finding is inconsistent, and it was not evident in other studies (33,35,41,64). In one study, CRP concentrations were less in dogs with obesity (34).

When assessing TG as an independent marker of ORMD in the dogs of the current study, positive associations with glucose, fructosamine, insulin and cholesterol were identified, whilst TG concentration was negatively associated with FFA. In people, plasma TG concentration is an independent predictor of MS (65). TG concentration, both independently and alongside other biochemical variables (such as insulin, hsCRP, adiponectin and HDL-cholesterol), can predict

304 a greater risk of type 2 DM and cardiovascular complications even in normal-weight humans,
305 and this analyte might be a more sensitive marker than the common definition of MS (66). Also,
306 studies in children have shown that a decrease in TG concentration following a low-fat diet,
307 was associated with healthier metabolic profiles even though no significant changes in BCS
308 were observed (67).

309 In one study, dogs with concurrent obesity were grouped according to the presence of metabolic
310 dysfunction using criteria similar to those of human MS (14); dogs were classified as having
311 so-called ORMD when BCS was between 7 and 9 and there were abnormalities in at least two
312 of the following: triglycerides, total cholesterol, systolic blood pressure and fasting plasma
313 glucose concentrations (14). The dogs classified in this way as ORMD did not have a greater
314 total fat mass than those without ORMD, but insulin concentration was greater and adiponectin
315 concentration was lower than in obese dogs not meeting the criteria for ORMD. This suggested
316 that the assessment of metabolic risk could help to classify dogs at risk of obesity-associated
317 comorbidities. According to the results of the current study, plasma TG concentration, used as
318 an only explanatory variable and correcting for the effect of the breed, were associated with
319 metabolic variables but not BCS, including greater glucose and fructosamine concentrations.
320 Of course, this finding should be interpreted in light of the fact that none of the dogs were in
321 obese body condition (BCS 8 to 9). To the author's knowledge, an association between TG and
322 glucose and fructosamine has not been previously reported in dogs, and might suggest an
323 association between increased glucose concentration and altered lipid metabolism.

Fructosamine is seldom researched in studies of human MS, but has been linked to cardiovascular outcomes, whilst its concentration increases with dyslipidaemia, including an association with triglyceride concentration (68).

Another important finding of the present study was the association between fasting TG and insulin concentrations which, to the best of the authors' knowledge, has not previously been described in healthy dogs. Increased TG concentrations are commonly associated with insulin resistance and type 2 DM in humans, and are considered to be the central feature of the dyslipidaemia that is present in these states (69–71). In dogs, it has been suggested that hypertriglyceridaemia is favoured by an increased supply of substrates to the liver (especially glucose and FFA) in insulin-resistant states (36).

In a study involving dogs with obesity, insulin concentration was less in a subgroup of persistently-hyperglycaemic dogs compared with obese dogs that were not persistently hyperglyacemic. Given that TG concentrations were also less in persistently-hyperglycemic obese dogs, it was hypothesised that TG might play a role in compensatory hyperinsulinaemia (and that a lack of TG could cause a decrease in insulin compensation for hyperglycaemia) (23). In a study involving healthy people without insulin resistance, there were differences between a control group, who responded to a TG infusion with an increase in insulin secretion, and a group with a family history of type 2 DM, who experienced a decrease in insulin secretion, as well as marked hepatic insulin resistance (72). This suggests that insulin responses vary greatly in different physiological states, and this should be considered in future studies

344 FFA concentrations tend to be greater in insulin-resistant humans, since insulin resistance leads
345 to a decreased insulin-mediated inhibition of lipolysis and, as a result, FFA concentrations tend
346 to increase (51). Furthermore, FFA are thought to be mechanistically involved in the
347 pathophysiology of obesity-induced insulin resistance (51). In studies involving dogs with
348 obesity, FFA concentrations increase and contribute to insulin resistance (36,37,46,69).
349 However, in the present study, FFA and TG concentrations were negatively associated. This
350 was unexpected given that increasing TG concentrations were also associated with greater
351 insulin, glucose and fructosamine concentrations, changes that are evocative of ORMD, and
352 ORMD is commonly associated with increased circulating FFA (51). One possible explanation
353 is that the severity of insulin resistance in the dogs of the present study was mild, or in the early
354 stages, primarily affecting the liver but not peripheral tissues (i.e., insulin is not able to inhibit
355 hepatic glucose production but it is effectively inhibiting lipolysis at the peripheral level). This
356 dissociation between hepatic and peripheral insulin resistance has previously been described
357 (73). An alternative explanation for the decreased FFA concentration, such as enhanced FFA
358 oxidation, seems unlikely given the other changes (increasing insulin, glucose, and
359 fructosamine), which are evocative of ORMD.

360 The positive association between serum TG and cholesterol has been previously described in
361 overweight dogs and was therefore expected.

362 Given that some breeds were overrepresented in the overweight category, the risk of
363 misinterpreting an effect of the breed with an effect of overweight was a concern. However, the

linear models used were corrected for the effect of the breed, and some effects were evident even in overweight dogs within single breeds. A sex effect was not assessed because, besides for Labrador retrievers, the study design meant that sex was covariate with breed. As a result, correction for the breed effect in the ANOVA and in the linear models would automatically correct for any effect of sex in most cases.

One limitation of the current study was that the BCS is an imperfect measure of body fat mass, and is influenced by many factors, including differences in breed morphologies and fat distribution (60). Further, subjectivity when assigning BCS might have led to the misclassification of some dogs, especially those examined at different centres. However, the same investigator was responsible for the assessment in each one of the five centres, and in all cases they were highly trained veterinarians that are expected to correlate greatly (74). Therefore, the associations found in the present study further support the utility of the BCS as a universal system to estimate fat excess. Different analysis combinations could have been used with BCS categories, but BCS 6 was chosen since it is a widely-accepted cut-off in every-day practice (7,17,18,60,75).

When assigning dogs to the overweight or lean categories, there was a predominance of dogs in the lean category in most breeds (≥80% in most breeds, except for CKCS, NF and LAB, which approached 50% in each category). Such an imbalance might have made it more difficult to identify statistically significant differences between lean and overweight categories within specific breeds that had a small number of overweight dogs. Of course, the limitations for

384 comparisons made between overweight and lean categories would not apply to comparisons
385 with TG concentrations, whose measurement is more accurate than the assessment of BCS.
386 This might have ensured a greater statistical power, explaining the increased number of
387 significant associations with the metabolic variables assessed.

388 Some may argue that an effect of the diet should have been included in the analyses. However,
389 this study was designed not to test for this parameter, but rather assess a cohort with
390 heterogeneous diets, representative of a client-owned population of dogs. Therefore, no
391 restriction was imposed in terms of diet, as long as the dogs had only access to a commercial
392 dry food diet and received no supplement during the three weeks preceding the analyses.

393 # Conclusions

394 Both BCS and serum TG concentrations were independently associated with changes in
395 markers of lipid and glucose metabolism in this large cohort of slightly overweight, otherwise
396 healthy dogs. Non obese, overweight (6-7) body condition was positively associated with
397 insulin and cholesterol concentrations whilst TG concentrations were positively associated with
398 cholesterol, insulin, glucose, and fructosamine concentrations, and negatively associated with
399 FFA concentrations. Therefore, both BCS and fasting TG concentration seem to be useful
400 markers of ORMD-related changes in dogs. Further analyses using more complex multivariate
401 models are needed to better characterize the interplay between these biochemical analytes.

402

References

1. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol [Internet]. 2011 Feb 21 [cited 2017 May 12];11(2):85–97. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21252989

2. Punthakee Z, Goldenberg R, Katz P. Definition, Classification and Diagnosis of Diabetes, Prediabetes and Metabolic Syndrome. Can J Diabetes [Internet]. 2018 Apr;42:S10–5. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1499267117308134

3. Verkest KR. Is the metabolic syndrome a useful clinical concept in dogs? A review of the evidence. Vet J [Internet]. 2014 Jan;199(1):24–30. Available from: http://dx.doi.org/10.1016/j.tvjl.2013.09.057

4. Chandler M, Cunningham S, Lund EM, Khanna C, Naramore R, Patel A, et al. Obesity and Associated Comorbidities in People and Companion Animals: A One Health Perspective. J Comp Pathol [Internet]. 2017 May [cited 2017 Nov 8];156(4):296–309. Available from: https://ac.els-cdn.com/S0021997517301226/1-s2.0-S0021997517301226-main.pdf?_tid=317ea704-c497-11e7-ba4e-00000aacb361&acdnat=1510154111_b97e24a33138d315df6b96e82e3ab679

5. Weeth LP. Other Risks/Possible Benefits of Obesity. Vet Clin North Am Small Anim Pract [Internet]. 2016 Sep [cited 2016 Dec 15];46(5):843–53. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0195561616300237

6. Clark M, Hoenig M. Metabolic Effects of Obesity and Its Interaction with Endocrine Diseases. Vet Clin North Am Small Anim Pract [Internet]. 2016 Sep [cited 2016 Dec 15];46(5):797–815. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0195561616300201

7. Kealy RD, Lawler DF, Ballam JM, Mantz SL, Biery DN, Greeley EH, et al. Effects of diet restriction on life span and age-related changes in dogs. J Am Vet Med Assoc [Internet]. 2002 May 1 [cited 2017 Jun 4];220(9):1315–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11991408

8. Lund EM, Armstrong PJ, Kirk CA, Klausner JS. Prevalence and Risk Factors for Obesity in Adult Dogs from Private US Veterinary Practices. J Appl Res Vet Med [Internet]. 2006;4(2):177–86. Available from: http://www.jarvm.com/articles/Vol4Iss2/Lund.pdf

9. Endenburg N, Soontararak S, Charoensuk C, van Lith HA. Quality of life and owner attitude to dog overweight and obesity in Thailand and the Netherlands. BMC Vet Res [Internet]. 2018 Dec 9;14(1):221. Available from: https://bmcvetres.biomedcentral.com/articles/10.1186/s12917-018-1531-z

10. Yam PS, Butowski CF, Chitty JL, Naughton G, Wiseman-Orr ML, Parkin T, et al. Impact of canine overweight and obesity on health-related quality of life. Prev Vet Med [Internet]. 2016 May [cited 2017 Sep 12];127:64–9. Available from: http://ac.els-

cdn.com/S0167587716300988/1-s2.0-S0167587716300988-main.pdf?_tid=f4c5ee4c-979d-11e7-a3e1-00000aacb35e&acdnat=1505209213_14e8f3f4af51412cc206bbdfcfb87449

11. Muñoz-Garach A, Cornejo-Pareja I, Tinahones F. Does Metabolically Healthy Obesity Exist? Nutrients [Internet]. 2016 Jun 1 [cited 2017 Jun 4];8(6):320. Available from: http://www.mdpi.com/2072-6643/8/6/320

12. Chang Y, Jung H-S, Yun KE, Cho J, Ahn J, Chung EC, et al. Metabolically healthy obesity is associated with an increased risk of diabetes independently of nonalcoholic fatty liver disease. Obesity [Internet]. 2016 Jul 30 [cited 2016 Aug 23];24(9):1996–2003. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27474900

13. Stefan N, Schick F, Häring H-U. Causes, Characteristics, and Consequences of Metabolically Unhealthy Normal Weight in Humans. Cell Metab [Internet]. 2017 Aug [cited 2018 Oct 24];26(2):292–300. Available from: http://dx.doi.org/10.1016/j.cmet.2017.07.008

14. Tvarijonaviciute A, Ceron JJ, Holden SL, Cuthbertson DJ, Biourge V, Morris PJ, et al. Obesity-related metabolic dysfunction in dogs: a comparison with human metabolic syndrome. BMC Vet Res [Internet]. 2012 Dec 28;8(1):147. Available from: http://www.biomedcentral.com/1746-6148/8/147

15. Tvarijonaviciute A, Ceron JJ, de Torre C, Ljubić BB, Holden SL, Queau Y, et al. Obese dogs with and without obesity-related metabolic dysfunction – a proteomic approach. BMC Vet Res [Internet]. 2016 Dec 20 [cited 2017 Jun 14];12(1):211. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5028949/pdf/12917_2016_Article_839.pdf

16. Lequarré A-S, Andersson L, André C, Fredholm M, Hitte C, Leeb T, et al. LUPA: A European initiative taking advantage of the canine genome architecture for unravelling complex disorders in both human and dogs. Vet J [Internet]. 2011 Aug [cited 2016 Aug 22];189(2):155–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1090023311002279

17. Laflamme D. Development and validation of a body condition score system for dogs. Canine Pract. 1997;22(4):10–5.

18. Brooks D, Churchill J, Fein K, Linder D, Michel KE, Tudor K, et al. 2014 AAHA Weight Management Guidelines for Dogs and Cats* †. J Am Anim Hosp Assoc [Internet]. 2014 Jan [cited 2017 Jun 28];50(1):1–11. Available from: https://www.aaha.org/public_documents/professional/guidelines/weight_management_guidelines.pdf

19. Diez M, Michaux C, Jeusette I, Baldwin P, Istasse L, Biourge V. Evolution of blood parameters during weight loss in experimental obese Beagle dogs. J Anim Physiol Anim Nutr (Berl) [Internet]. 2004 Apr;88(3–4):166–71. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1439-0396.2003.00474.x

20. German AJ, Hervera M, Hunter L, Holden SL, Morris PJ, Biourge V, et al. Improvement in insulin resistance and reduction in plasma inflammatory adipokines

after weight loss in obese dogs. Domest Anim Endocrinol [Internet]. 2009 Nov;37(4):214–26. Available from: https://linkinghub.elsevier.com/retrieve/pii/S073972400900071X

21. Tvarijonaviciute A, Tecles F, Martinez-Subiela S, Ceron JJ. Effect of weight loss on inflammatory biomarkers in obese dogs. Vet J [Internet]. 2012 Aug;193(2):570–2. Available from: http://search.proquest.com/docview/914408438?accountid=13042%5Cnhttp://oxfordsfx.hosted.exlibrisgroup.com/oxford?url_ver=Z39.88-2004&rft_val_fmt=info:ofi/fmt:kev:mtx:dissertation&genre=dissertations+&+theses&sid=ProQ:ProQuest+Dissertations+&+Theses+Global

22. Kim SP, Catalano KJ, Hsu IR, Chiu JD, Richey JM, Bergman RN. Nocturnal free fatty acids are uniquely elevated in the longitudinal development of diet-induced insulin resistance and hyperinsulinemia. Am J Physiol - Endocrinol Metab [Internet]. 2007;292(6):1590–8. Available from: http://ajpendo.physiology.org/content/292/6/E1590.short

23. Verkest KR, Rand JS, Fleeman LM, Morton JM. Spontaneously obese dogs exhibit greater postprandial glucose, triglyceride, and insulin concentrations than lean dogs. Domest Anim Endocrinol [Internet]. 2012 Feb [cited 2016 Mar 7];42(2):103–12. Available from: http://www.sciencedirect.com/science/article/pii/S0739724011001494

24. Söder J, Wernersson S, Hagman R, Karlsson I, Malmlöf K, Höglund K. Metabolic and Hormonal Response to a Feed-challenge Test in Lean and Overweight Dogs. J Vet Intern Med [Internet]. 2016 Mar 29;30(2):574–82. Available from: http://doi.wiley.com/10.1111/jvim.13830

25. Hoenig M. Comparative Aspects of Human, Canine, and Feline Obesity and Factors Predicting Progression to Diabetes. Vet Sci [Internet]. 2014 Aug 21;1(2):121–35. Available from: http://www.mdpi.com/2306-7381/1/2/121/

26. Chan JM, Rimm EB, Colditz GA, Stampfer MJ, Willett WC. Obesity, Fat Distribution, and Weight Gain as Risk Factors for Clinical Diabetes in Men. Diabetes Care [Internet]. 1994 Sep 1;17(9):961–9. Available from: https://diabetesjournals.org/care/article/17/9/961/17890/Obesity-Fat-Distribution-and-Weight-Gain-as-Risk

27. Veiga APM, Santos AP, Santos WIM, González FHD. Fructosamine as a Tool on the Evaluation of Insulin Resistant Obese Dogs. ARC J Anim Vet Sci [Internet]. 2016 [cited 2017 Feb 27];2(2):2455–518. Available from: https://www.arcjournals.org/pdfs/ajavs/v2-i2/4.pdf

28. Broussolle C, Tricot F, Garcia I, Orgiazzi J, Revol A. Evaluation of the fructosamine test in obesity: consequences for the assessment of past glycemic control in diabetes. Clin Biochem [Internet]. 1991 Apr [cited 2017 Jun 2];24(2):203–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2040093

29. Woo J, Cockram C, Lau E, Chan A, Swaminathan R. Influence of obesity on plasma fructosamine concentration. Clin Chem [Internet]. 1992 Nov [cited 2017 Jun 2];38(11):2190–2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1424109

30. Zoran DL. Obesity in Dogs and Cats: A Metabolic and Endocrine Disorder. Vet Clin North Am Small Anim Pract [Internet]. 2010 Mar;40(2):221–39. Available from: http://dx.doi.org/10.1016/j.cvsm.2009.10.009

31. Aronson D, Bartha P, Zinder O, Kerner A, Markiewicz W, Avizohar O, et al. Obesity is the major determinant of elevated C-reactive protein in subjects with the metabolic syndrome. Int J Obes [Internet]. 2004 May 2 [cited 2019 May 28];28(5):674–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14993913

32. Radakovich LB, Truelove MP, Pannone SC, Olver CS, Santangelo KS. Clinically healthy overweight and obese dogs differ from lean controls in select CBC and serum biochemistry values. Vet Clin Pathol [Internet]. 2017 Jun;46(2):221–6. Available from: https://onlinelibrary.wiley.com/doi/10.1111/vcp.12468

33. Tvarijonaviciute A, Martinez S, Gutierrez A, Ceron JJ, Tecles F. Serum acute phase proteins concentrations in dogs during experimentally short-term induced overweight. A preliminary study. Res Vet Sci [Internet]. 2011 Feb [cited 2017 Jun 5];90(1):31–4. Available from: https://vpn.gw.ulg.ac.be/S0034528810001773/,DanaInfo=ac.els-cdn.com+1-s2.0-S0034528810001773-main.pdf?_tid=15a6737c-49d6-11e7-aecc-00000aacb35e&acdnat=1496657129_c7263074d33e46efaa1c64dd4e93d81a

34. Veiga APM, Price CA, de Oliveira ST, dos Santos AP, Campos R, Barbosa PR, et al. Association of canine obesity with reduced serum levels of C-reactive protein. J Vet Diagnostic Investig [Internet]. 2008 Mar 1 [cited 2017 May 11];20(2):224–8. Available from: http://journals.sagepub.com/doi/pdf/10.1177/104063870802000214

35. Tropf M, Nelson OL, Lee PM, Weng HY. Cardiac and Metabolic Variables in Obese Dogs. J Vet Intern Med [Internet]. 2017 Jul;31(4):1000–7. Available from: http://doi.wiley.com/10.1111/jvim.14775%0Ahttp://www.ncbi.nlm.nih.gov/pubmed/28608635

36. Bailhache E, Nguyen P, Krempf M, Siliart B, Magot T, Ouguerram K. Lipoproteins abnormalities in obese insulin-resistant dogs. Metabolism [Internet]. 2003 May;52(5):559–64. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0026049503000234

37. Gayet C, Bailhache E, Dumon H, Martin L, Siliart B, Nguyen P. Insulin resistance and changes in plasma concentration of TNFalpha, IGF1, and NEFA in dogs during weight gain and obesity. J Anim Physiol Anim Nutr (Berl) [Internet]. 2004 Apr;88(3–4):157–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15059241

38. Jeusette IC, Lhoest ET, Istasse LP, Diez MO. Influence of obesity on plasma lipid and lipoprotein concentrations in dogs. Am J Vet Res [Internet]. 2005 Jan 1 [cited 2016 Aug 8];66(1):81–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15691040

39. Tribuddharatana T, Kongpiromchean Y, Sribhen K, Sribhen C. Biochemical alterations and their relationships with the metabolic syndrome components in canine obesity. Kasetsart J - Nat Sci [Internet]. 2011 [cited 2017 Jun 4];45(4):622–8. Available from: http://www.thaiscience.info/journals/Article/TKJN/10898335.pdf

40. José Lahm Cardoso M, Fagnani R, Zaghi Cavalcante C, de Souza Zanutto M, Júnior AZ, Holsback da Silveira Fertonani L, et al. Blood Pressure, Serum Glucose,

Cholesterol, and Triglycerides in Dogs with Different Body Scores. Vet Med Int [Internet]. 2016;2016:1–7. Available from: https://www.hindawi.com/journals/vmi/2016/8675283/

41. Adolphe JL, Silver TI, Childs H, Drew MD, Weber LP. Short-term obesity results in detrimental metabolic and cardiovascular changes that may not be reversed with weight loss in an obese dog model. Br J Nutr [Internet]. 2014 Aug 30;112(04):647–56. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24877650

42. Geffré A, Concordet D, Braun J-P, Trumel C. Reference Value Advisor: a new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel. Vet Clin Pathol [Internet]. 2011 Mar;40(1):107–12. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1939-165X.2011.00287.x

43. Friedrichs KR, Harr KE, Freeman KP, Szladovits B, Walton RM, Barnhart KF, et al. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. Vet Clin Pathol. 2012 Dec;41(4):441–53.

44. R Development Core Team: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. 2014.

45. Peña C, Suárez L, Bautista I, Montoya JA, Juste MC. Relationship between analytic values and canine obesity. J Anim Physiol Anim Nutr (Berl) [Internet]. 2008 Jun;92(3):324–5. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1439-0396.2007.00786.x

46. Bailhache E, Ouguerram K, Gayet C, Krempf M, Siliart B, Magot T, et al. An insulin-resistant hypertriglyceridaemic normotensive obese dog model: assessment of insulin resistance by the euglycaemic hyperinsulinaemic clamp in combination with the stable isotope technique. J Anim Physiol Anim Nutr (Berl) [Internet]. 2003;87(3–4):86–95. Available from: http://doi.wiley.com/10.1046/j.1439-0396.2003.00419.x

47. Jeusette IC, Detilleux J, Shibata H, Saito M, Honjoh T, Delobel A, et al. Effects of chronic obesity and weight loss on plasma ghrelin and leptin concentrations in dogs. Res Vet Sci. 2005;79(2):169–75.

48. Lee S, Kweon O-K, Kim WH. Increased Leptin and Leptin Receptor Expression in Dogs With Gallbladder Mucocele. J Vet Intern Med [Internet]. 2017 Jan [cited 2017 Nov 29];31(1):36–42. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5259632/pdf/JVIM-31-36.pdf

49. Parker VJ, Freeman LM. Association between Body Condition and Survival in Dogs with Acquired Chronic Kidney Disease. J Vet Intern Med [Internet]. 2011 Nov;25(6):1306–11. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1939-1676.2011.00805.x

50. Myers DG, Bach PJ, Schreiber FB. Normative and Informational Effects of Group Interaction*. Sociometry. 1974;37(2):275–86.

51. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature. 2006.

608 52. Anagnostis P, Athyros VG, Tziomalos K, Karagiannis A, Mikhailidis DP. The
609 Pathogenetic Role of Cortisol in the Metabolic Syndrome: A Hypothesis. J Clin
610 Endocrinol Metab [Internet]. 2009 Aug 1;94(8):2692–701. Available from:
611 https://academic.oup.com/jcem/article/94/8/2692/2596309

612 53. Nelson RW, Reusch CE. Animal models of disease: classification and etiology of
613 diabetes in dogs and cats. J Endocrinol. 2014;222(3).

614 54. Gilor C, Niessen SJM, Furrow E, DiBartola SP. What's in a Name? Classification of
615 Diabetes Mellitus in Veterinary Medicine and Why It Matters. J Vet Intern Med
616 [Internet]. 2016 [cited 2018 Feb 14];30(4):927–40. Available from:
617 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5108445/pdf/JVIM-30-0927.pdf

618 55. Banfield. State of Pet Health 2014 Report. 2016;

619 56. Mattin M, O'Neill D, Church D, McGreevy PD, Thomson PC, Brodbelt D. An
620 epidemiological study of diabetes mellitus in dogs attending first opinion practice in
621 the UK. Vet Rec [Internet]. 2014 Apr;174(14):349–349. Available from:
622 http://doi.wiley.com/10.1136/vr.101950

623 57. Klinkenberg H, Sallander MH, Hedhammar A. Feeding, Exercise, and Weight
624 Identified as Risk Factors in Canine Diabetes Mellitus. J Nutr [Internet]. 2006 Jul
625 1;136(7):1985S-1987S. Available from:
626 https://academic.oup.com/jn/article/136/7/1985S/4664763

627 58. Pöppl AG, de Carvalho GLC, Vivian IF, Corbellini LG, González FHD. Canine
628 diabetes mellitus risk factors: A matched case-control study. Res Vet Sci [Internet].
629 2017 [cited 2017 Dec 12];114:469–73. Available from: https://ac.els-
630 cdn.com/S0034528817303156/1-s2.0-S0034528817303156-main.pdf?_tid=c5f1f458-
631 df28-11e7-831d-
632 00000aab0f6c&acdnat=1513075367_33935133eb97f7dd7cb02b3b9f49aa02

633 59. Lawler DF, Larson BT, Ballam JM, Smith GK, Biery DN, Evans RH, et al. Diet
634 restriction and ageing in the dog: major observations over two decades. Br J Nutr
635 [Internet]. 2008 Apr 6 [cited 2016 Aug 31];99(04):793–805. Available from:
636 http://www.journals.cambridge.org/abstract_S0007114507871686

637 60. Jeusette I, Greco D, Aquino F, Detilleux J, Peterson M, Romano V, et al. Effect of
638 breed on body composition and comparison between various methods to estimate body
639 composition in dogs. Res Vet Sci [Internet]. 2010;88(2):227–32. Available from:
640 http://dx.doi.org/10.1016/j.rvsc.2009.07.009

641 61. Kim SP, Ellmerer M, Kirkman EL, Bergman RN. beta-Cell "rest" accompanies
642 reduced first-pass hepatic insulin extraction in the insulin-resistant, fat-fed canine
643 model. AJP Endocrinol Metab [Internet]. 2007 Jan 30 [cited 2017 Jun
644 12];292(6):E1581–9. Available from:
645 http://ajpendo.physiology.org/content/ajpendo/292/6/E1581.full.pdf

646 62. Raffan E, Dennis RJ, O'Donovan CJ, Becker JM, Scott RA, Smith SP, et al. A
647 Deletion in the Canine POMC Gene Is Associated with Weight and Appetite in
648 Obesity-Prone Labrador Retriever Dogs. Cell Metab [Internet]. 2016 May;23(5):893–
649 900. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1550413116301632

650 63. Colliard L, Ancel J, Benet J-J, Paragon B-M, Blanchard G. Risk Factors for Obesity in
651 Dogs in France. J Nutr [Internet]. 2006 Jul 1;136(7):1951S-1954S. Available from:
652 https://academic.oup.com/jn/article/136/7/1951S/4664731

653 64. Leclerc L, Thorin C, Flanagan J, Biourge V, Serisier S, Nguyen P. Higher neonatal
654 growth rate and body condition score at 7 months are predictive factors of obesity in
655 adult female Beagle dogs. BMC Vet Res. 2017;13(1):1–13.

656 65. Yuan G, Al-Shali KZ, Hegele RA. Hypertriglyceridemia: Its etiology, effects and
657 treatment [Internet]. Vol. 176, CMAJ. 2007 [cited 2017 Jun 2]. p. 1113–20. Available
658 from:
659 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1839776/pdf/20070410s00019p1113.p
660 df

661 66. Eckel N, Mühlenbruch K, Meidtner K, Boeing H, Stefan N, Schulze MB.
662 Characterization of metabolically unhealthy normal-weight individuals: Risk factors
663 and their associations with type 2 diabetes. Metabolism [Internet]. 2015 [cited 2017
664 Aug 24];64(8):862–71. Available from:
665 http://www.metabolismjournal.com/article/S0026-0495(15)00087-6/pdf

666 67. Cambuli VM, Musiu MC, Incani M, Paderi M, Serpe R, Marras V, et al. Assessment of
667 adiponectin and leptin as biomarkers of positive metabolic outcomes after lifestyle
668 intervention in overweight and obese children. J Clin Endocrinol Metab.
669 2008;93(8):3051–7.

670 68. Peng YF, Wei YS. The relationships between serum fructosamine concentrations and
671 lipid profiles in community-dwelling adults. Sci Rep [Internet]. 2017;7(1):3–7.
672 Available from: http://dx.doi.org/10.1038/s41598-017-07287-5

673 69. Ginsberg HN, Zhang Y-L, Hernandez-Ono A. Regulation of plasma triglycerides in
674 insulin resistance and diabetes. Arch Med Res [Internet]. 2005 [cited 2017 Jun
675 3];36(3):232–40. Available from:
676 https://vpn.gw.ulg.ac.be/S0188440905000068/,DanaInfo=ac.els-cdn.com+1-s2.0-
677 S0188440905000068-main.pdf?_tid=298aca28-489b-11e7-a180-
678 00000aacb362&acdnat=1496521871_771cdf524bc71d83099f9276c36fcafc

679 70. Glueck CJ, Khan NA, Umar M, Uppal MS, Ahmed W, Morrison JA, et al. Insulin
680 Resistance and Triglycerides. J Investig Med [Internet]. 2009 Dec 1 [cited 2019 May
681 26];57(8):874–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19809367

682 71. Hoffman RP. Increased fasting triglyceride levels are associated with hepatic insulin
683 resistance in caucasian but not African-American adolescents. Diabetes Care.
684 2006;29(6):1402–4.

685 72. Kashyap S, Belfort R, Gastaldelli A, Pratipanawatr T, Berria R, Pratipanawatr W, et al.
686 A Sustained Increase in Plasma Free Fatty Acids Impairs Insulin Secretion in
687 Nondiabetic Subjects Genetically Predisposed to Develop Type 2 Diabetes. Diabetes
688 [Internet]. 2003 Oct 1;52(10):2461–74. Available from:
689 http://www.ncbi.nlm.nih.gov/pubmed/14514628

690 73. Kim SP, Ellmerer M, Van Citters GW, Bergman RN. Primacy of hepatic insulin
691 resistance in the development of the metabolic syndrome induced by an isocaloric

moderate-fat diet in the dog. Diabetes [Internet]. 2003 Oct [cited 2016 Aug 5];52(10):2453–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14514627

74. German AJ, Holden SL, Moxham GL, Holmes KL, Hackett RM, Rawlings JM. A simple, reliable tool for owners to assess the body condition of their dog or cat. J Nutr [Internet]. 2006 [cited 2016 Feb 16];136:2031S-2033S. Available from: http://www.mendeley.com/catalog/simple-reliable-tool-owners-assess-body-condition-dog-cat/

75. German AJ, Holden SL, Morris PJ, Biourge V. Comparison of a bioimpedance monitor with dual-energy x-ray absorptiometry for noninvasive estimation of percentage body fat in dogs. Am J Vet Res [Internet]. 2010 Apr;71(4):393–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20367047

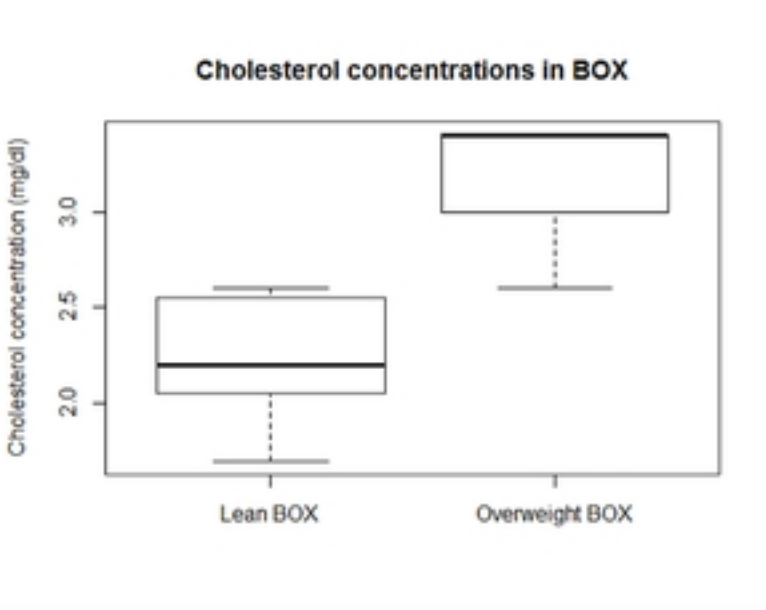
Acknowledgements

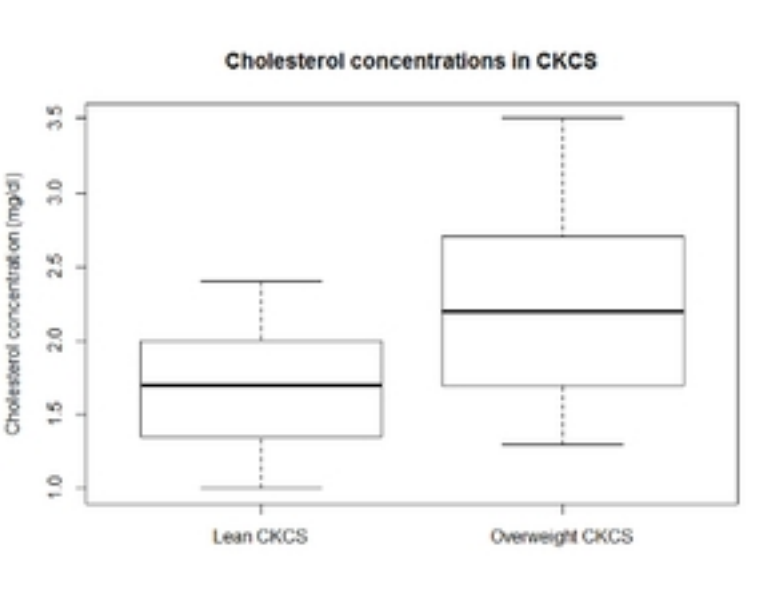
Belgian police and its contribution to the collection of dogs.

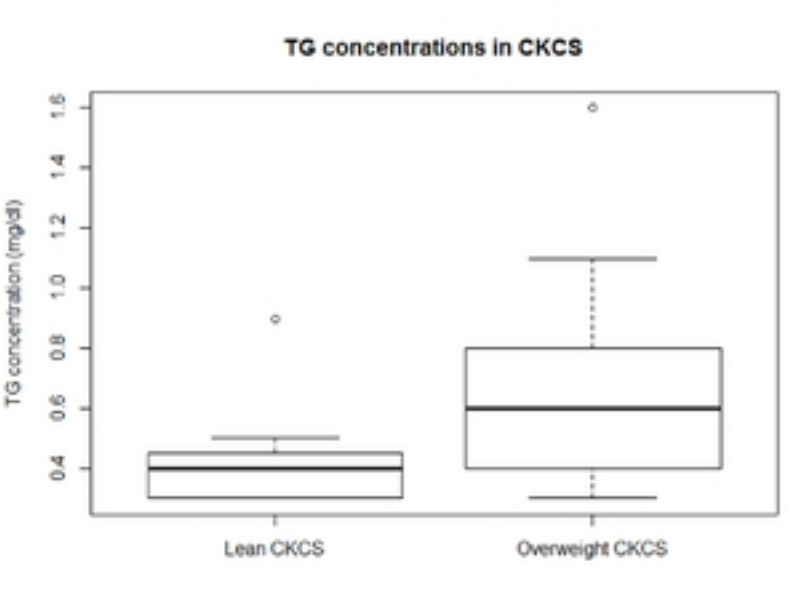
Supporting information

S1 File. The protocol.

S2 File. The dataset.


Cholesterol concentrations in BOX
Cholesterol concentration (mg/dl)
2.0
2.5
3.0
Lean BOX
Overweight BOX


Cholesterol concentrations in CKCS
Cholesterol concentration (mg/dl)
1.0
1.5
2.0
2.5
3.0
3.5
Lean CKCS
Overweight CKCS


TG concentrations in CKCS
TG concentration (mg/dl)
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Lean CKCS
Overweight CKCS

Figure