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ORIGINAL ARTICLE

Recovery of insulin sensitivity and optimal body composition after rapid weight loss in obese dogs fed a high-protein medium-carbohydrate diet

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Summary

This study investigated the effects of an experimental high-protein medium-carbohydrate diet (protein level, 46% metabolizable energy, ME). First, postprandial plasma glucose and insulin kinetics were determined in steady-state overweight/obese Beagle dogs (28%–41% excess body weight) for an experimental high-protein medium-carbohydrate diet (protein level, 46% ME) and a commercial high-carbohydrate medium-protein diet (protein level, 24%ME) in obese dogs. Secondly, all the dogs were included in a weight loss programme. They were fed the high-protein medium-carbohydrate diet, and the energy allocation was gradually reduced until they reached their optimal body weight. Insulin sensitivity and body composition were evaluated before and after weight loss using a euglycaemic–hyperinsulinaemic clamp and the deuterium oxide dilution technique respectively. For statistical analysis, linear mixed effect models were used with a significance level of 5%. Postprandial plasma glucose and insulin concentrations were substantially lower with the high-protein medium-carbohydrate diet than the high-carbohydrate medium-protein diet. These differences can be explained mainly by the difference in carbohydrate content between the two diets. Energy restriction (35% lower energy intake than in the obese state) resulted in a 2.23 ± 0.05% loss in body weight/week, and the dogs reached their optimal body weight in 12–16 weeks. Weight loss was associated with a significant increase in insulin sensitivity. The high-protein medium-carbohydrate diet allowed fat-free mass preservation despite a relatively high rate of weekly weight loss. The increase in insulin sensitivity indicated improved control of carbohydrate metabolism, possible due to weight loss and to the nature of the diet. Thus, a high-protein medium-carbohydrate diet is a good nutritional solution for managing the weight of overweight dogs. This diet may improve glycaemic control, which could be beneficial for preventing or managing impaired glucose tolerance in obese dogs and for safe and successful weight loss while preserving lean body mass.

Keywords overweight, starch, glucose, metabolism, clamp, lean body mass

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Introduction

Surveys of obesity in the canine population that have been performed worldwide indicate that the prevalence of canine obesity has been increasing for years (Colliard et al., 2006; Laflamme, 2006; Courcier et al., 2010; Mao et al., 2013). Obese dogs are those with a body condition score of 4/5 or 5/5 according to the WSAVA Nutritional Assessment Guidelines (WSAVA Nutritional Assessment Guidelines Task Force Members, 2011). According to the Association for Pet Obesity Prevention (http://www.petobesityprevention.org), as many as 53.8% of US dogs would have been overweight or obese in 2015. Canine obesity is associated with important metabolic and hormonal changes (Gayet et al., 2004; Clark and Hoenig, 2016), such as impaired glucose tolerance, reduced sensitivity to insulin (German et al., 2010b; Laflamme, 2011) and dyslipidaemia (Bailhache et al., 2003; Verkest et al., 2012). Moreover, obesity is considered a significant risk factor in the development of chronic diseases, such as renal disease, heart disease and cancer (Thengchaisri et al., 2014; Weeth, 2016).

With respect to glucose intolerance and reduced insulin sensitivity, approaches that use low glycaemic index diets have been tested to improve weight and metabolic outcomes (Mitsuhashi et al., 2012). Foods with low glycaemic index may also reduce oxidative stress and protect the cardiovascular system in dogs (Adolphe et al., 2012). The glycaemic index was developed to rank carbohydrate-containing foods according to their effects on blood glucose levels in humans (Jenkins et al., 1981). Rapidly available glucose (RAG) is defined as the amount of glucose released *in vitro* from a food during the first 20 min of incubation with adequate enzymes, and it has been shown to be related to the glycaemic response in human studies (Englyst et al., 1999). In rats, both plasma glucose and insulin responses were positively correlated to the rate of hydrolysis with α-amylase *in vitro* (Holm et al., 1988). However, a more recent human study showed that two carbohydrate sources with similar RAG content elicited similar glycaemic responses but different insulin responses (Al-Mssallem et al., 2011). Thus, predicting the effects of a diet on postprandial plasma glucose and insulin kinetics is not easy, and field trials in target species are necessary to accurately evaluate the *in vivo* responses.

In dogs, postprandial glucose kinetics are mainly influenced by the amount of starch in the diet (Nguyen et al., 1994). Nevertheless, many factors other than available carbohydrate level affect postprandial glycaemia and insulinaemia, including starch source and structure, macronutrient content and source (Kendall et al., 2006). In particular, it has been shown, in humans, that high protein intake increases insulin secretion (Gulliford et al., 1989), and moreover that different sources of protein may lead to different insulin responses (Nuttall and Gannon, 1990).

Compared to the starch level, the protein level has a limited impact on postprandial glycaemia, but there has long been interest in the use of high-protein diets for weight loss. Orthopaedic or other surgical procedures may require weight loss, either for causal reasons (orthopaedic procedures) or because obesity makes anaesthetic management more difficult or is a risk factor for surgical site infections (Love and Cline, 2015). Therefore, high-energy restriction may be chosen when there is a medical indication for rapid weight loss. In dogs, energy restriction is suggested for losses of 1%–2% of the initial body weight (BW) per week (Nguyen and Diez, 2010). In all cases, it takes months to lose the excess 25%–30% of excess BW that is, the weight over the optimal BW. Although the literature is not entirely conclusive on this point, in general, the risk of losing lean body mass is higher with a higher rate of weight loss. In such cases, attention should be paid to the composition of the diet and to the provision of essential nutrients, including protein. Lower lean body mass loss was observed during weight loss when dogs were given a high-protein diet (crude protein, CP: 39% metabolizable energy, ME) compared to a medium-protein diet (CP: 20%ME) (Hannah, 1999). In a previous study we conducted in obese dogs, we showed that a high-protein diet (CP: 41%ME) allowed the conservation of lean body mass despite a high rate of weight loss (2.6% loss per week) (Blanchard et al., 2004). More recently, two other high-protein diets (CP: ~40%ME) were also shown to allow safe weight loss in dogs, but with a lower rate of weight loss of 0.7%–1.0% per week (German et al., 2010a).

In the present study, we compared two diets with protein levels of 46%ME or 24%ME. Although the terms do not have widely accepted definitions, these diets can be considered high-protein and medium-protein diets, respectively, in accordance with terminology used in previous studies and in accordance with the minimum FEDIAF recommendation (2016) for maintenance (CP: 18%ME), which is the lowest acceptable level.

The objectives of our research were twofold. Study 1 compared the postprandial glycaemic and insulinaemic kinetics induced by a high-protein medium-carbohydrate (HP-MC) diet vs. a high-carbohydrate medium-protein (HC-MP) diet in overweight/obese dogs. Study 2 assessed the effects of the HP-MC diet on body composition and insulin sensitivity in overweight/obese dogs, energy restricted to induce rapid weight loss.

Materials and methods

Animals

Nine adult overweight/obese Beagle dogs (five neutered males and four spayed females) aged 3.2 ± 0.4 years were included in these studies. The experimental protocols adhered to European Union guidelines and followed the requirements for animal welfare of the French Ministry of Agriculture. This work was approved by the Animal Use and Care Advisory (Comité d'Éthique en Expérimentation Animale des Pays de la Loire; approval number 00281.02). The dogs were housed individually in an air-conditioned environment. The room temperature was maintained between 19 °C and 24 °C. The dogs were housed in the same room with permanent visual contact with each other to prevent isolation stress. As a group, they went outdoors daily to interact and exercise for a limited and controlled period of time. Water was freely available at all times.

Before the two studies began, dogs with an optimal BW of 13.1 ± 0.7 kg were fed a high-energy diet and developed obesity. Given the same diet at ~150 kcal (628 kJ) ME/kg optimal $BW^{0.75}$, the dogs were maintained in an overweight or obese state for 30 weeks.

At the beginning of Study 2, the dogs' mean BW was 17.6 ± 0.9 kg. The mean percentage of excess BW was 34% (range: 28%–41%), and the dogs' body condition score (BCS) was 7 (n = 1) or 8 (n = 8) on a nine-point scale (Laflamme, 1997). BW and BCS were monitored and recorded weekly.

Diets

Two diets were used in Study 1: a commercial HC-MP medium-energy diet, and a nutritionally balanced experimental HP-MC low-energy diet that was not commercially available at the time of the study. The chemical composition of each diet is presented in Table 1. In Study 2, only the HP-MC diet was used.

Table 1 Nutritional characteristics of an experimental high-protein medium-carbohydrate (HP-MC) diet and a commercial high-carbohydrate medium-protein (HC-MP) diet: chemical analysis (as fed) and contribution of macronutrients to metabolizable energy (%ME). The two diets were both used in an acute feeding trial (Study 1), and the HP-MC diet was also used in a long-term feeding trial (Study 2)

	HP-MC diet	HC-MP diet
Moisture (%)	7.0	7.4
Crude protein (%)	37.5	22.4
Crude fat (%)	11.4	13.5
Nitrogen-free extract (%)	23.0	41.6
Crude fibre (%)	13.8	10.8
Starch (%)	13.4	31.9
Total sugars (%)	0.7	0.3
Insoluble fibre (%)	22.8	17.0
Soluble fibre (%)	1.6	0.8
Ash (%)	7.3	4.3
Metabolizable energy* (kcal/100 g)	293	332
Metabolizable energy* (kJ/100 g)	1230	1390
Protein (%ME)*	46	23
Starch (%ME)*	19	41
Fat (%ME)*	31	36

*in vivo determination.
HP-MC diet contained dehydrated pork and poultry proteins, lignocellulose, potato starch, hydrolysed animal protein, animal fat, field bean hulls, minerals, dried beet pulp, psyllium fibre, fructo-oligosaccharides, chitosan, chondroitin sulphate, pasteurized Lactobacillus acidophilus, vitamins.
HC-MP contained maize, dehydrated poultry proteins, rice, cellulose, animal fat, hydrolysed animal protein, pea fibre, dried whole egg, vegetable oil, linseed, minerals, vitamins.

In vitro measurement of RAG in the diets

The measurement of RAG in the two diets was performed by Agrobio (Vezin Le Coquet, France). The *in vitro* technique (Englyst et al., 1999) is based on HPLC measurement of the glucose released from the food after a 20-min incubation with digestive enzymes (pepsin, pancreatin, invertase and amyloglucosidase).

Study design

In Study 1, eight dogs, in stable overweight or obese state, were subjected, in a crossover design, to postprandial plasma glucose and insulin kinetics assays while they were fed either the HP-MC diet or the HC-MP diet. Between the two kinetics assays, the dogs were fed, for 1 week, the diet used for the development of obesity. In Study 2, nine dogs were subjected to a weight loss programme with the HP-MC diet. The target was the recovery of optimal BW. The dogs were fed once daily. The initial food allocation was 120 kcal (503 kJ) ME/kg optimal $BW^{0.75}$, which represents an approximate 20% decrease relative to the previous diet. The target weekly weight loss rate was 2%–2.5% (compared to the BW of the previous week). The food allowance was decreased by 10% as many times as necessary when an insufficient change in BW was observed over a 2-week period. The duration of the study was not predetermined; it lasted until each dog reached its optimal BW. Body composition and insulin sensitivity were evaluated before the weight loss intervention and when the target weight was achieved (see below for details).

Postprandial kinetics and biochemical analyses (Study 1)

On the morning of the plasma glucose and insulin kinetics assays (i.e., the test days) and after a 24-h food deprivation period, eight overweight/obese dogs were fed either the HP-MC or HC-MP diet in a single meal. This comprised 544 kJ/130 kcal ME/kg optimal $BW^{0.75}$, which was the nearest value to their consumption in the stable obese state, and was in the range of FEDIAF recommendations (2016) for BW maintenance in adult dogs. The postprandial kinetics assays were performed using a crossover design in that during the week between the two test days, the dogs were fed the diet that was used for the development of obesity. All dogs consumed their meal in less than

10 min. Jugular vein blood samples were collected before the meal, immediately after the meal, and then 10, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300 and 360 min after the end of the meal. Blood glucose concentrations were determined in whole blood using a portable blood glucose meter (Alphatrak, Abbott Laboratories, IL, USA) that was validated for use in dogs (Kang et al., 2015). The heparinized blood samples were immediately refrigerated on ice and centrifuged (2200 × *g*, 10 min, 4 °C). The plasma was collected and stored at –20 °C until analysis. Plasma insulin was determined by radioimmunoassay (Insulin IRMA kit, Beckman Coulter, Prague, Czech Republic).

Euglycaemic–hyperinsulinaemic clamp (Study 2)

A 3-h euglycaemic–hyperinsulinaemic clamp was used to estimate insulin sensitivity before and after weight loss with the HP-MC diet in seven 24-h unfed dogs as described previously (Bailhache et al., 2003).

Body composition (Study 2)

Body composition was determined three times: before the period of weight gain, in the stable overweight/obese state and after the weight loss programme. Total body water (TBW) was assessed using an isotopic dilution of ^2H_2O. After a 24-h food deprivation period, dogs were confined to metabolism cages. Water was withheld, allowing body water equilibration from 2 h before to 4 h after the tracer injection. Dogs were injected subcutaneously (0.50 g/kg BW) with physiological saline (9 g NaCl/L) prepared with ^2H_2O (99.9% $^2H/H$, Eurisotop, Gif-sur-Yvette, France). Venous blood samples were obtained prior to and 4 h after injection of the tracer. EDTA plasma was stored at −20 °C in sealed vials until analysis. Deuterium enrichment ($^2H/H$) in plasma water was determined by Fourier transform infrared spectroscopy on a Vector 22-type spectroscope (Brüker SA, Wissembourg, France) (Ferrier et al., 2002). TBW was determined from the dilution space of the isotope. Fat-free mass (FFM) was calculated as the TBW/hydration rate of FFM using a canine-specific hydration rate of 0.744 (Harrison et al., 1936).

Data analysis

All data were expressed as means ± standard deviation. To take into account the crossover design of the postprandial kinetics study, the two diets were compared using a linear mixed effects model analysis and using Tukey's tests. For an accurate analysis of the repeated measures design, a mixed effects model was used for body composition and insulin sensitivity index comparisons. Analyses were performed in R software (R Core Team, 2013) using the nlme and multcomp packages. The nlme package that was provided by the Rcore team contains all of the functions used for analysis in a mixed effects model (linear or nonlinear). We considered $p < 0.05$ to indicate a significant difference between data sets.

Results

Study 1

Postprandial glycaemic and insulinaemic kinetics

The kinetics of the glucose (Fig. 1a) and insulin (Fig. 1b) responses were different between the two tested diets. The glucose peak appeared earlier for the HP-MC diet than for the HC-MP diet. The maximum glucose increase did not differ significantly, but the area under the glucose response curve was approximately double for the HC-MP diet compared to the HP-MC diet (44 ± 35 g.min/l vs. 21 ± 10 g.min/l, p = 0.044). The maximum plasma insulin response was significantly lower for the HP-MC diet than for the HC-MP diet (32 ± 11 μU/ml vs. 68 ± 39 μU/ml, p = 0.031, Table 2). The incremental area under the insulin response curve, which could be considered a first approximation of the total insulin secretion, was approximately threefold higher for the HC-MP diet than for the HP-MC diet (12 400 ± 6400 μU.min/ml vs. 3600 ± 2500 μU.min/ml, p = 0.004). The average postprandial insulinaemia was significantly higher for the HC-MP diet (47.1 ± 13.2 μU/ml vs. 30.3 ± 9.3 μU/ml, p = 0.002).

Determination of RAG in foods

The estimated values of the *in vitro* glycaemic index were 20 and 44 (on a scale of 0–100) for the HP-MC and HC-MP diets respectively.

Study 2

BW, BCS and characteristics of the weight loss period

Dogs reached their target weight, which corresponded to their optimal BW (13.1 ± 0.7 kg and a BCS of 5), after 12–16 weeks (mean, 14.3 ± 1.4 weeks). The mean weight loss rate was 2.23 ± 0.05% per week. The mean daily energy allowance was 98 ± 3 kcal (410 ± 13 kJ) ME/optimal $BW^{0.75}$ over the entire weight loss period. The mean daily consumption of protein was 12.9 ± 2.5 g/kg optimal $BW^{0.75}$.

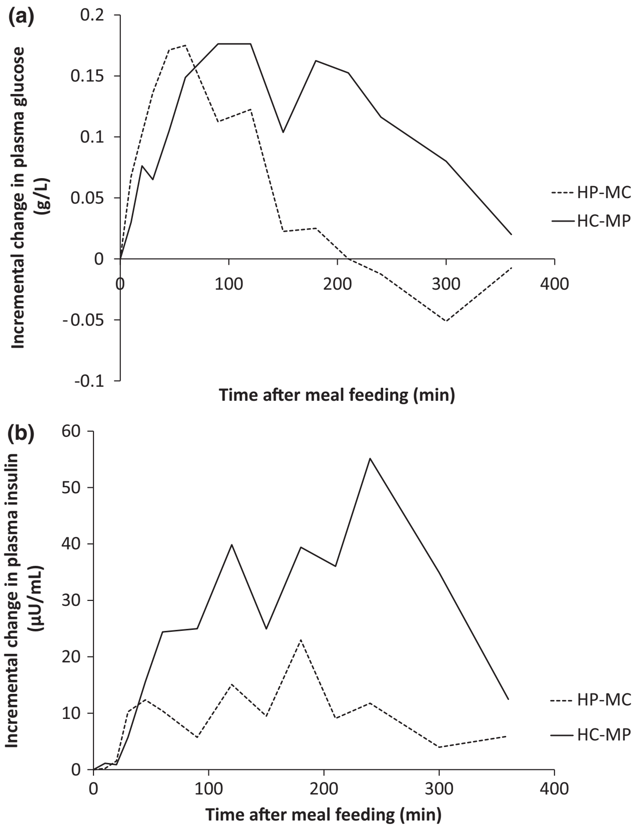

Fig. 1 Postprandial incremental changes in plasma glucose concentration (a) and insulin concentration (b) from time 0 values in obese dogs fed a high-protein medium-carbohydrate (HP-MC) diet or a high-carbohydrate medium-protein (HC-MP) diet (Study 1; *n* = 8). Values are expressed as means.

Table 2 Plasma glucose and insulin response characteristics (mean, maximum increments and area under the curves) in obese dogs after a meal-feeding test using a high-protein medium-carbohydrate (HP-MC) diet or a high-carbohydrate medium-protein (HC-MP) diet (Study 1; *n* = 8). Values are expressed as means ± SD

	HP-MC diet	HC-MP diet	p
Mean postprandial glycaemia (g/l)	1.05 ± 0.10	1.11 ± 0.13	ns
Mean postprandial insulinaemia (μU/ml)	30.3 ± 9.3	47.1 ± 13.2	**
AUCG (g x min/l)	21 ± 10	44 ± 35	*
AUCI (μU x min/ml)	3600 ± 2500	12 400 ± 6700	**
Maximum glucose increment (g/l)	0.23 ± 0.08	0.27 ± 0.16	ns
Maximum insulin increment (μU/ml)	32 ± 11	68 ± 39	*

AUCG, area under the curve for glucose; AUCI, area under the curve for insulin. *p < 0.05, **p < 0.01, ns no significant difference between the two tested diets. Values are expressed as mean ± SD, where *n* = 8 dogs.

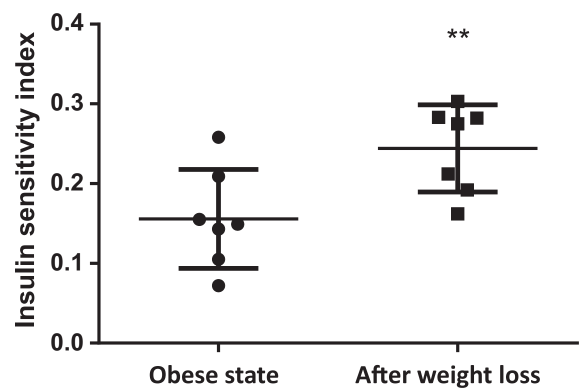

Fig. 2 Insulin sensitivity as measured in Study 2 by the euglycaemic–hyperinsulinaemic clamp method in seven dogs before and after weight loss with a high-protein medium-carbohydrate (HP-MC) diet. **p < 0.01 between lean and obese groups. Means ± SD are indicated.

Insulin sensitivity

The plasma insulin and glucose concentrations in the unfed state were not significantly different in the obese dogs than in the dogs at the end of the weight loss period (data not shown). As Fig. 2 illustrates, insulin sensitivity was higher after weight loss (0.24 ± 0.05 vs. 0.15 ± 0.06, p = 0.006). The euglycaemic–hyperinsulinaemic clamp failed in two dogs,

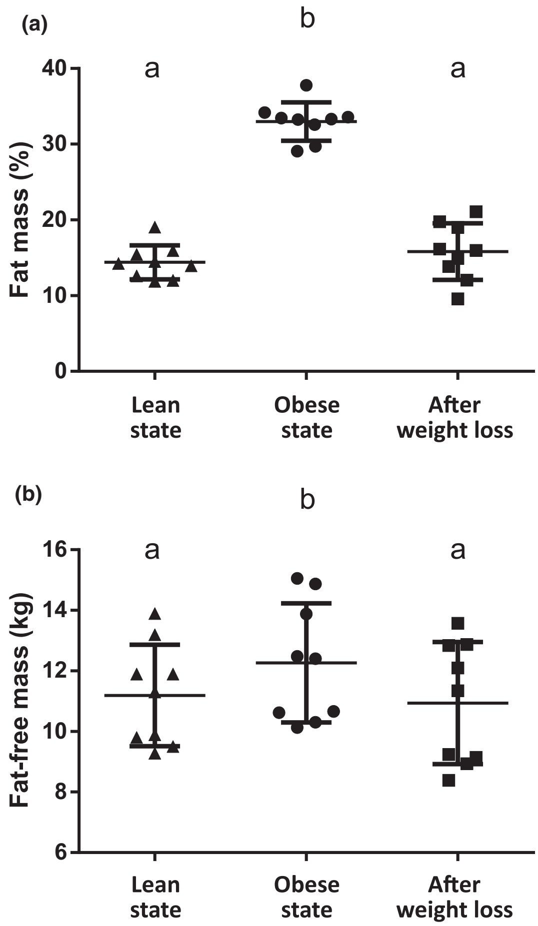

Fig. 3 Body composition of the dogs as determined in Study 2 using deuterium oxide dilution to assess (a) the fat mass (FM) as a percentage of body weight (BW) (%FM) and (b) the fat-free mass (FFM) in kg, at an optimal time (in the lean state before weight gain), in the obese state and after weight loss with a high-protein medium-carbohydrate (HP-MC) diet. Means ± SD are indicated; $n = 9$. Means with different superscripts differ ($p < 0.0001$).

showing unexpected and unexplained changes in plasma glucose despite constant insulin and glucose infusion rates.

Body composition

The body composition results are presented in Fig. 3. Weight loss resulted in a significant decrease in fat mass (FM) and FFM ($p < 0.0001$). The body composition after weight loss was similar to the body composition assessed at the optimal BW.

Discussion

The first aim of the study was to compare the effects of two types of meals, that is, the HP-MC and HC-MP diets, on postprandial glycaemic and insulinaemic responses in overweight/obese dogs. Starch content, which was between 0.4 and 52.7 g/100 g dry matter in the tested diets, is the primary determinant of postprandial glucose in healthy dogs (Nguyen et al., 1998). Elliott et al. (2012) showed that a restricted carbohydrate diet facilitated the control of blood glucose levels in healthy dogs: a medium-carbohydrate (25%ME) high-protein (43%ME) diet resulted in lower peak and postprandial glucose concentrations compared to a common adult maintenance diet (high carbohydrate, 55%ME; medium protein, 22%ME). Despite different levels of carbohydrates in the two tested diets in Study 1 (Starch: 19%ME vs. 40%ME) and different *in vitro* RAG concentrations (20 for the HP-MC diet vs. 44 for the HC-MP diet), we observed no significant differences in the mean postprandial plasma glucose concentration or in the maximum glucose increase. However, the HP-MC diet led to significant differences in insulinaemic responses (lower maximum increment, lower mean, lower AUCI). Our results are in agreement with those of Hewson-Hughes et al. (2011), who reported that there were minimal changes in plasma glucose concentration whatever the level of starch in the diet and that the increase in insulin concentration was lower in healthy dogs fed a low-starch diet (9.5%) compared to a medium-starch (23%) or high-starch (31.7%) diet. We hypothesize that the results we obtained on the test days would be the same as those resulting from usual and daily consumption.

There is currently no standardized method for evaluating the glycaemic index in non-human species. It would have been interesting to use the same method as Adolphe et al. (2012). For a given food that has 10 g of available carbohydrate, the glycaemic index was calculated by expressing the area under the curve for glucose as a percentage of the mean response to the glucose control (a 20% glucose solution providing 10 g of glucose) that was consumed by the same dog. In our study, the two diets were compared without normalizing the quantity of digestible carbohydrates.

Dietary fibre, another macronutrient, has been evaluated for its potential benefits in glucose supply regulation in dogs. Variations in fibre intake from 3.2 to 39.1 g of total dietary fibre/100 g DM do not significantly influence postprandial glucose and insulin responses in healthy dogs (Nguyen et al., 1998). In our investigation, although the sources and levels of fibre were different, the different levels of starch rather than the quantity and quality of the fibre might explain the results.

The question of whether high postprandial insulinaemia and glycaemia have deleterious impacts in obese dogs still has no clear answer. Adolphe et al. (2014) suggested that a diet that would significantly reduce the postprandial insulin response, even without weight loss, might improve the metabolic profile of dogs. With regard to postprandial responses, the HP-MC diet appears to be more appropriate for overweight dogs. However, obesity is associated with numerous diseases (cancer, heart disease, chronic kidney disease), and the healthiest solution may be weight loss.

The second objective of this study was to investigate the effect of a diet with high-protein (46%ME) and medium-carbohydrate content (19%ME) during weight loss on FFM preservation. This diet was optimized for the nutritional management of reduction of excessive BW in obese dogs, which were thus the target animals.

The first determinant of weight lost is energy restriction: energy intake must be lower than energy expenditure. The dogs' energy consumption during the weight loss period (98 ± 3 kcal (410 ± 13 kJ) ME/kg $BW^{0.75}$) may appear high, as it is in the range of the FEDIAF recommendations (2016) for maintenance in adult dogs (95–130 kcal (397-544 kJ) ME/kg $BW^{0.75}$, depending on sexual status and physical activity). However, the animals included in this study were kennel dogs, and their usual food consumption provided 150 kcal (628 kJ) ME/kg $BW^{0.75}$, as determined to maintain their optimal BW. The diet used to fight obesity has to be specifically balanced to cover all nutritional requirements while preserving lean mass. In the present study, dogs fed a high-protein diet quickly recovered their optimal weight, and their body composition was similar to before they gained weight. The higher absolute FFM in the obese state may be related to the increase in the mass of some organs and the mass of body fluids, as reported in humans (Bosy-Westphal et al., 2009; Pourhassan et al., 2014).

Although the term "high-protein diet" is used, it should be kept in mind that dogs require an absolute protein provision rather than a high dietary protein level and that the protein requirement of dogs remains a grey zone, even for a maintenance diet (minimal vs. optimal, National Research Council (2006) vs. FEDIAF (The European Pet Food Industry Federation) (2016) recommendations). In our study, the mean daily consumption of protein was 12.9 ± 2.5 g/kg optimal $BW^{0.75}$. The efficacy of a high-protein, low-carbohydrate, low-energy diet (CP 47.5%DM, i.e., approximately 63%ME, starch 5.3% DM, 11.6 kJ/g as fed) for weight loss has been shown in obese Beagles, as this diet allowed rapid and safe weight loss without altering the lean mass (Diez et al., 2002). The mean daily consumption of protein in that study was 15.4 g/kg optimal $BW^{0.75}$. Blanchard et al. (2004) showed the same results with another high-protein, medium-carbohydrate, low-energy diet (CP 40%ME, carbohydrate 30%ME, 10.5 kJ/g as fed), and the mean daily consumption of protein was as low as 6.3 g/kg optimal $BW^{0.75}$. In the study by Hannah (1999), a high-protein diet (CP 39%ME) resulted in a lower loss of lean tissue than a medium-protein one (CP 20%ME), while the loss of lean mass with an intermediary diet (CP: 30%ME) was not significantly different from that determined by the two other diets. Unfortunately, this study did not state how much protein the dogs consumed nor could that be calculated.

Taken together, these data may look somewhat inconsistent, but this is probably not the case. Indeed, like our study, these studies aimed to show that diets that are formulated for weight loss programmes must have a higher protein-to-energy ratio than maintenance diets in order to restrict the energy allowance while ensuring that the dogs' protein requirement is met. These studies may also have intended to show that dogs receiving an energy-restricted diet that subsequently lose weight could benefit from a higher protein provision, as was recently shown in older people for whom the protein provision (in g/kg) should be twice as high as the maintenance requirement (Weijs and Wolfe, 2016). This would not probably be the case in dogs, at least in the mentioned studies. Indeed, if a minimal protein provision is needed to counteract protein turnover and thereby ensure the preservation of lean body mass, once this goal is reached, there is probably nothing more to expect from a higher protein allowance. There are a few ways to explain differences between the dog studies and the cited human studies. First, the human studies were conducted in older people in whom relative anabolic resistance has been described (Moore et al., 2015). Second, the usual daily protein allowance in dogs is already higher than their strict protein requirement. Indeed, the usual allowance is approximately three times (2.5 vs. 0.83 g/kg) that of humans, and about three times (~5 vs. 1.8 g/kg $BW^{0.75}$) the minimum determined in dog nitrogen balance studies (Kendall et al., 1982). In these respects, the allowance (6.3 g/kg optimal $BW^{0.75}$) in the experiment in the Blanchard et al. (2004) study was probably high enough to ensure preservation of lean mass (compared to the previous lean state), and it is not surprising that higher protein provisions gave a similar result. Accordingly, if the dietary protein level must be higher in diets intended for weight loss than in maintenance diets (according to FEDIAF recommendation (2016)), there is no evidence to substantiate the need for an absolute higher protein provision during weight loss than during maintenance in dogs, given that the usual recommendation is already higher than the strict requirement.

A high-protein diet is not only efficient in terms of preserving FFM, but it is also in keeping with some dogs' carnivorous nutritional traits. A study that investigated macronutrient selection in domestic dogs showed that when dogs had a choice of foods with variable protein, fat and carbohydrate content, they chose a diet with approximately one-third of the energy derived from protein, 7% from carbohydrates and more than 60% of energy from fat (Hewson-Hughes et al., 2013). Wolves, which are pack-hunting progenitors of the domestic dog, are adaptive and true carnivores. Their protein requirement is much higher than that of dogs (Bosch et al., 2015). Even though dogs have adapted to a starch-rich diet during domestication, they still share numerous traits with true carnivores (Bosch et al., 2015). A high-protein diet may more closely reflect dogs' ancestral dietary pattern. Moreover, rapid feeding, which is an adaptation to scavenging, is also a trait in this dietary pattern, and dogs that retain this tendency can rapidly become obese (Bradshaw, 2006). As humans regulate the intake of protein energy (PE) more strongly than non-protein energy (nPE), it has been proposed that the rise in obesity may have resulted partly from a shift towards diets with reduced PE:nPE ratios (Raubenheimer et al., 2015). High-protein diets could therefore be beneficial in dogs in terms of the prevention of overweight and, at the same time, be in accordance with the ancestral dietary pattern of the dogs.

Lastly, the insulin sensitivity index showed that obese dogs are more insulin-sensitive after they lose weight. Our results showed that the changes in glucose metabolism observed in dogs that were suffering from primary obesity due to excess food intake are reversible. This improvement may be due mainly to weight loss *per se*, as demonstrated previously (German et al., 2009). Another study that used a control diet during weight loss could clarify the relative importance of weight loss and diet composition on the improvement of insulin sensitivity.

Our study showed that a HP-MC diet may be a good nutritional solution for safe weight loss in overweight/obese dogs, as it allowed rapid weight loss with preservation of lean body mass. Treating obesity could improve insulin sensitivity. A diet with a protein-to-carbohydrate ratio of 1.6 and a protein-to-energy ratio of 112 g MP/Mcal ME (28 g MP/MJ ME) was associated with lower postprandial glucose and insulin concentrations than a diet with a protein-to-carbohydrate ratio of 0.5 and a protein-to-energy ratio of 61 g MP/Mcal ME (15 g MP/MJ ME). Such a HP-MC diet may help in glycaemic control, which could be advantageous for the prevention or management of impaired glucose tolerance or diabetes mellitus in obese dogs.

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