The effects of *ad libitum* feeding of low- or highpalatable feed on the physical activity, bodyweight and feeding patterns of domestic cats.

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Abstract

Obesity has become one of the most common disorders in companion animals with a 25% prevalence in some western countries. Obese cats have a much higher risk to develop serious illnesses like diabetes mellitus and hepatic lipidosis. Several risk factors have been known to facilitate the development of obesity, for example: neutering or underestimation of body condition by the owner. This study will focus on 3, still widely discussed, possible risk factors: an ad libitum feeding regime, physical activity and palatability. The aim will be to look for effects of *ad libitum* feeding with a low- (LP) or high-palatable (HP) diet on the physical activity, bodyweight and feeding patterns of domestic cats. And if the results of this study can help owners with controlling their cat's bodyweight. Sixteen domestic shorthair cats were used. Each week two cats started the 3 week long course. The 1st and 3rd week were control weeks. The 2nd week was the test week where the cats had access to their personal feeding station with ad libitum either LP or HP food in it and feeding parameters were measured. Activity was monitored during all 3 weeks. Food intake was higher for the HP diet when compared to the LP diet (P=0.043). And number of meals increased as the days progressed (P=0.013). Total feeding time, average meal time and intake/requirement ratio had possible trends but variation between cats was too big. Both the HP and LP diet showed a lower activity count compared to the first control week (P=0.01 and P<0.001 respectively), the LP diet was also lower than control week 2 (P=0.003). The type of week (Control week 1, Control week 2, HP diet or LP diet), day of week and clock hour all had a significant interaction on activity counts. The lower activity count was probably due to a lower food anticipatory activity, which alternatively is the reason for a higher activity in meal fed cats. With the results of this study it was not possible to formulate a satisfactory answer to our research question. A subsequent more elaborative study in which physical activity, weight changes, and ad libitum feeding with a suitable adaptation period beforehand, should be studied so a definitive conclusion about the matter can hopefully be made.

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1. Introduction

In recent years overweight and obesity in companion animals has become more and more prevalent, especially in the western world. Overweight and obesity have been defined as 'a condition of positive energy balance and excessive adipose tissue formation with adverse effects on morbidity and mortality' [Crane, 1991]. When pets have a 20% excess of their ideal body weight it is agreed that the animals are obese [Laflamme, 2012]. With an excess body weight between 0-20% they are classified as overweight. Several studies have been conducted to study the prevalence of overweight in cats. The United states is the leading country in regards to feline overweight with 59% of all cats being overweight or obese according to a nationwide survey in 2012 [Calabash, March 12, 2013]. In Great Britain, France, Perth Australia and New Zealand the percentages are 11.5%, 7.8%, 18.9% and 27%, respectively [Robertson, 1999; Colliard et al., 2009; Courcier et al., 2012; Cave et al., 2012].

Overweight/obese cats have a higher risk of suffering from several obesity-related health problems, such as: diabetes mellitus, hepatic lipidosis, urinary tract diseases, lameness and dermatopathies [Scarlett and Donoghue, 1998; Lund et al., 2005]. The risk for development of diabetes mellitus type 2 through decrease in insulin activity increases 2-fold in overweight cats and 4-fold in obese cats [Hoenig et al., 2007].

Commonly, animals become overweight or obese when their energy intake exceeds their energy requirement for a longer period of time. However, a large number of other factors also seem to play a role in this anabolic process. Most studies performed agree on several factors being bigger risks for developing overweight. These factors are: male neutered cats, cross-bred or mixed breed cats, cats of middle age and underestimation of body condition by the owner [Crane, 1991; Robertson, 1999; Allan et al., 2000; Lund et al., 2005; Kienzle and Bergler, 2006; Colliard et al., 2009; Slingerland et al., 2009; Cave et al., 2012; Courcier et al., 2012; Fallman et al., 2014]. Other factors, which some authors accept and others reject, are: living in a single or two-cat household, no dog living in the household, indoor confinement, a human child living in the same house, feeding premium or therapeutic foods, feeding fresh meat or fish, feeding on an *ad libitum* basis or with 1 or more meals. Of all these factors, underestimation of body condition by the owner seems to be the most important for practical intervention. From a study in Paris [Colliard et al., 2009] it was shown that owners misinterpret their cat's body condition regularly. Often considering normal weight cats as underweight and overweight cats as normal. This may make the owners feed their cats more to encourage 'underweight' cats to gain weight, thereby making them more likely to become overweight when the cats get older. Not just what is 'normal', but also what is 'healthy', will change the owner's perception of their cat's body condition. An owner will be less likely to define their pet as obese if they do not believe that their pet's current body condition is associated with disease. Laflamme et al. [2008] contacted 1104 pet owners in the US and Australia to determine owners attitudes towards their pets. Thirty-two percent of these owners classified their pet as obese but only 0.8% believed that it was a health problem. As a consequence, these owners were not motivated at all to make their pets lose weight.

Of all the factors mentioned above, the dietary factors are still widely researched as no consensus has been reached yet if they help with the development or prevention of getting overweight. One of these dietary factors for obesity is giving your pet premium or therapeutic foods [Lund et al., 2005].

These foods are purchased from pet stores or veterinary practices and generally have a higher caloric density than the regular dry supermarket pet foods. The conclusion therefore, was that owners were more likely to overfeed their pets which led to overweight. However, Colliard [2009] and Cave [2012] did not reach the same conclusion. Therapeutic foods are prescribed specific medical problems, which sometimes includes obesity. These foods were excluded from the studies of Colliard [2009] and Cave [2012] because it was not clear whether the obesity in the animals receiving these foods were cause or effect. With only the premium foods being used in the study, no evidence was found that cats receiving premium foods were more often overweight or obese.

Another risk factor associated with feeding is the feeding regimens owners apply. Feeding your cat *ad libitum* or giving your cat 1 or more meals per day is still a topic of discussion. Some researchers say that *ad libitum* feeding is a risk factor for the development of overweight [Russell et al., 2000; Harper et al., 2001; Kienzle and Bergler, 2006], others do not [Allan et al., 2000; Colliard et al., 2009; Cave et al., 2012] and still others say that *ad libitum* feeding even gives a lower BCS as a result [Robertson, 1999]. Russel et al. [2000] found that cats fed *ad libitum* canned food had a significantly higher BCS than cats which were being meal-fed. However, there was no significant difference in BCS in cats which were fed with dry food with either an *ad libitum* or meal-feeding regimen. Cave et al. [2012] also researched the effect of *ad libitum* feeding and frequency of feeding on the weight of cats. They hypothesized that the cats of owners who put a large amount of food in a bowl and ensured that the bowl was never empty, thus meeting the criterion for *ad libitum* feeding, were eating less because the food became stale and unpalatable. On the other hand, when owners often provide small amounts of food and keep the bowl full it was hypothesized that cats were more encouraged to eat because the food was fresh. However, neither the frequency of feeding nor the *ad libitum* feeding regimen was a statistically significant risk factor for obesity.

Ad libitum feeding shares similarities with the natural feeding behaviour of cats in the wild. Feral cats try to maintain a set weight by regulating how much they eat. This means that when there is an abundance of food, as with ad libitum feeding, the cats eat frequently but only small amounts each time [Surgess and Hurley, 2007]. Feral cats feed mostly on small mammals [MacDonald et al., 1984; Biro et al., 2005; Plantinga et al., 2011]. An average mouse provides approximately 30 kcal (126 kJ). This is about 8% of the daily energy requirement for a cat [Mugford, 1977]. This means that the cat needs around 12 of these one-mouse meals per day to meet its energy requirement. This is in accordance to other studies where cats, given a free food-choice have been shown to eat 7-20 times a day to acquire their daily energy intake [Kanarek, 1975; Kaufman et al., 1980; Kane et al., 1981b]. Kanarek [1975] went further and studied the effects on meal duration and meal frequency when changing from a regular chow (3.4 kcal/g) to an energy diluted diet (2.7 kcal/g). Both the average meal durations as the average meal frequency increased when given the diluted diet compared to the normal chow. Although the meal duration and frequency increased, the cats consumed less food per meal with the diluted diet. The total food intake however remained constant. Kanarek [1975] concluded that the energy density is one of the factors that determines meal patterns of cats. But that food consumption stays constant rather than increasing intake to compensate for a decrease in caloric density of the diet. This conclusion was supported by Hirsch et al. [1978]. However, this is in contrast to the findings of Castonguay [1981] and Kane et al. [1981b]. They were able to show that cats quickly adjusted their daily food intake to compensate for different caloric densities in different diets to maintain a constant caloric intake. Kane et al. suggested that the previous negative results [Kanarek, 1975; Hirsch et al., 1978] were possibly the result of using high levels of unpalatable

diluents (cellulose and kaolin). The effect of palatable diets, which are nutritionally equivalent, on feeding patterns of cats is not extensively researched. Peachey and Harper [2002] studied the meal patterns of 2 groups of cats (old vs. young) when given canned food and food enriched with vegetable oil or beef tallow. The cats showed no difference in meal duration or number of meals. The only differences were the lower intakes of the vegetable oil enriched diets compared to the beef tallow diet. This is in accordance with the results of Kane et al. [1981a] who demonstrated that beef tallow enriched diets were preferred above vegetable oil enriched diets when given access to both. But whether or not this preference is also responsible for a significant increase in food intake compared to regular food is not identified. In rats however, Sunday et al. [1983] had some interesting findings. Nutritionally equivalent diets were used which only differed in palatability. When offered sequentially these diets did not induce different meal patterns. However, when offered simultaneously the more palatable diet was eaten more frequently, in larger amounts and in a faster rate than the less-palatable diet. Furthermore, the meal patterns for the palatable diet was roughly the same when given sequentially or simultaneously whereas the meal patterns were reduced for the less-palatable diet. Thus, it appeared that the appeal was reduced for the less-palatable diet rather than enhanced for the palatable diet when given simultaneously.

A decline in physical activity has also been reported to be a risk factor for developing obesity and feline diabetes [Slingerland et al., 2009]. This decline in physical activity is mostly due to indoor confinement of the cats by the owners. It was hypothesized by Deng et al.[2011] that increasing the feeding frequency would encourage cats to get food and therefore changing their activity patterns and increasing their overall daily activity. Their conclusion was that there was no activity difference between two-time and four-time feeding patterns. However, it was stated that a multiple-meal feeding strategy may increase overall activity of cats compared to a one-meal feeding strategy. In a follow-up study, Deng et al. [2014] studied the effects of feeding 1, 2, 4, or a random number of meals per day, on the voluntary physical activity of 10 cats. One-meal fed cats showed a lower daily activity level than 4-meal-fed and random-meal-fed cats and also the food anticipatory activity was lower in 1-meal-fed cats than multiple-meal-fed cats. Thus, increasing the feeding frequency appears to promote physical activity and may aid with weight management in cats. However, Deng et al. [2014] only spoke of different meal feeding regimens, an *ad libitum* feeding regimen was not mentioned in either article.

Another factor to be considered is the palatability of the food. There is evidence in humans [Malandrino and Capristo, 2012] and in rats [Levin and Dunn-Meynell, 2002] that palatable food positively reinforces eating, thus increasing the likelihood to develop obesity. Scientific studies on this subject in cats however, are lacking. The palatability of food depends on a variety of factors including aroma, taste, temperature, texture and consistency [Bradshaw et al., 1996]. Cats usually prefer soft moist food with roughly the same water content as meat (70-85%). Yet most cats also readily accept semi-moist or dry food. A fatty coating applied on the outside of dry kibble can further enhance palatability through the softening of the texture rather than improving the flavor. The neuronal units with which cats are able to taste flavors respond positively to 'sweet' amino acids (proline, cysteine, ornithine, lysine, histidine and alanine) and respond negatively to 'bitter' amino acids (arginine, isoleucine, phenylalanine and tryptophan)[Bradshaw et al., 1996]. Despite of the preference for 'sweet' amino acids, the neuronal units are not stimulated by sugars [Zaghini and Biagi, 2005]. The 'sweet' amino acids are found mostly in fresh meat making it more attractive to cats and they are used in commercial cat diets to help with food acceptance. To summarize, obesity in pet cats is a big problem. Preventing rather than treating the obesity is priority. Several factors can be associated with the development of obesity. This study researched the effects of an *ad libitum* feeding method, with either a low- or high-palatable food, on the physical activity, bodyweight and feeding patterns of domestic cats. Both activity and meal patterns are important factors in controlling the weight of cats. Identifying if *ad libitum* feeding with different palatable foods will change activity and meal patterns, and subsequently weight of domestic cats, might help cat owners with controlling their pet's weight.

2. Materials and Methods

Experimental design

The physical activity and feeding characteristics of animals receiving an *ad libitum* relatively low palatable (LP) or high palatable (HP) diet were monitored. The study was conducted in pairs in a parallel design with one cat only receiving one test diet. The cats were paired up according to sex and then assigned to either the HP or LP diet using a randomisation procedure in Excel. The experiment length was 3 weeks for each cat. In week 1 cats were fed in the regular meal schedule of twice daily with regular food and physical activity was monitored using Actical Activity Monitors[®] to measure base activity level. Week 2 was the test week. During this week the 2 cats received *ad libitum* an experimental diet which differed only in palatability (LP or HP), they had access to 2 feeding stations in which their food was placed. During this *ad libitum* test week the amount of food and number of station visits were monitored using a weighing scale and data logger built into the feeding station. Physical activity was also monitored to measure any changes. In week 3 cats returned to their normal 2-meal feeding schedule with regular food and physical activity was measured. On day 1 and 5 of every week the BW and BCS was monitored.

Feeding station

The two feeding stations were made of Perspex on a wooden platform and was 90x40x60 cm in size and both were placed in the cats' group room. The test-cat could enter and leave the feeding station any time during the test week through the Microchip ID System (Sure-Flap®) in the cat flap which was installed in the front of the Perspex box. As the subdermal chips did not respond on the cat flap the cats wore a chip on their collar to gain access. To prevent other cats from intruding, the passage of the cat flap was extended 10 cm with special attachments and narrowed using a piece of Perspex and duct tape. Also a Perspex wall was installed inside the box in order to make the cats curve their way towards the food. This way, the cat flap system closed faster. However, this did not stop the cats on several occasions to enter the feeding station with more than 1 cat. To keep track of this, cameras were installed in the 4th week of the study. Whenever it was seen that more than 1 cat or a different cat entered the feeding station the food intake data was adjusted or not used at all.

In a previous study, the cats were already introduced with getting fed inside the stations. Most of the cats recognized the station and hardly needed any training. Those cats that did need training were trained by luring them through the cat flap using food rewards after which they had access to a whole bowl of food. This was repeated until they got through the cat flap on their own initiative. The first day of data collecting in the test week was therefore not used in statistical analyses. The feeding area in the box could be accessed by the researchers through a door which was located at the top of the box and on the side of the box. The *ad libitum* fed cats received their food in a food bowl in the feeding station. The food bowl was set on a digital weighing scale which was incorporated into the wooden part of the station. This scale (the A&D EK-2000i) was connected to a AD-1688 portable data logger to continuously register any weight loss of the food bowl. With a registration interval of 20 seconds it was possible to continuously register a period of more than 24 hours. The data were collected every day.

Animals and housing

Sixteen domestic shorthair cats from Wageningen University were used for this study. The male (n=8) and female (n=8) cats had a mean age (\pm SD) of respectively 2.21 (\pm 0.12) and 2.19 (\pm 0.16) y, BW of 2.65 (\pm 0.14) and 3.20 (\pm 0.14) kg and BCS of C and B at the start of the study.

The animals remained in their familiar facilities with the 8 males and 8 females housed in separate rooms. The facility had an indoor and outdoor part where the cats could play, jump and climb and also has several resting possibilities. It had a natural day-and-night cycle where the lights switched off at 20:00. The temperature fluctuated between 19°C and 24°C. When not in their test week, the cats were fed twice daily (7:30 hour and 15:30 hour) and had *ad libitum* access to clean drinking water. In the 4th week into the study, cameras were installed in both rooms to monitor irregularities with data logger data.

Diets

During week 1 and week 3, the cats were fed twice daily at 7:30 hour and 15:30 hour with a commercial dry extruded diet (Perfect Fit In-home, Mars Petcare, Verden, Germany). For the test week, two diets from a previous study were used: an uncoated dry extruded diet (Vobra Special Petfoods, Veghel, the Netherlands) was coated with 3% poultry fat and either 0% (LP) or 2% (HP) of commercial palatant (AFB International, Nuland, the Netherlands) (See table 1 for the chemical compositions of the diets. It was stored in the freezer in the time between this study and the previous study. To familiarize the cats with the new diet, each cat was fed their respective diet on at least 5 occasions before the start of their test week. The energy requirement (ER) of individual cats was calculated using:

ER = 53.7 kcal/kg BW^{$^{1.061}$}, when BW < 3.0 kg;

ER = 46.8 kcal/kg BW^{$^{1.115}$}, when 3.0 kg < BW < 5.5 kg;

Based on the calculated ER and the metabolic energy (ME) of the regular chow, the food quantity for each cat was determined in weeks 1 and 3.

During the *ad libitum* test week (week 2) the food bowl was filled up to circa 110 g whenever it was close to being empty and at the end of the day before everyone left the building. The weight of the bowl was monitored before and after every fill up.

	1				
Diets	Protein, %	Fat, %	CF, %	NFE, %	ME, kcal/100g
N	41.0	14.5	3.5	23.0	372.8
LP	29.0	15.0	1.5	40.5	374.9
HP	29.4	15.1	1.5	40.5	374.7

N = normal dry extruded diet (Perfect Fit In-home); LP = low-palatability test diet produced without commercial palatant; HP = high-palatability test diet which was produced with 2% of commercial palatant; CF = crude fibre; NFE = nitrogen-free extract(NFE = Dry matter-protein-fat-crude fibre-ash contents); ME = metabolic energy (calculate procedures (NRC2006a): kcal ME = kcal DE - (0.77 x g protein); kcal DE = (kcal GE x energy digestibility)/100; energy digestibility (100%) = 87.9 - (0.88 x % CF in DM); and kcal GE = (5.7 x g protein) + (9.4 x g fat) + [4.1 x (g NFE + g crude fibre)]).

Feeding pattern parameters

Several parameters were examined to study the feeding patterns of the cats. The duration of the average daily feeding time, number of meals and food intake was calculated using the weighing scale data. Average daily eating time is the total amount of time per day that cats spend eating. The average eating time per meal was calculated by dividing the daily eating time with the number of meals. Food intake is shown as grams of food per day. Metabolic food intake is shown as grams of food per kg of metabolic bodyweight to account for the differences in bodyweight of the different cats. Energy intake was calculated by multiplying the metabolic energy of the diets with the daily food intake. Energy intake/requirement ratio was calculated by dividing daily energy intake with the basal energy requirement of the cats. The basal energy requirement for each cat can be seen in Index I.

Physical activity measurement

The physical activity was monitored with the use of the Actical Activity Monitor[®] (Mini Mitter, Bend, OR, USA). The Actical was 28×27×10 mm in size and weighs 17 grams and contains omnidirectional sensors that are capable of registering the intensity and duration of movements [Deng et al., 2011]. This accelerometer was incorporated into a collar. The cats wore this for a period of 3 consecutive weeks, from Monday morning 11:00 hour till Monday morning 11:00 hour three weeks later.

During the time of activity monitoring a list was present on the door of the cat facility to keep record of events that would interfere with the activity monitoring. For example when the collars, with the Acticals on them, came off. The data were collected using the Actical 3.0 program. The Actical software presented each interval count (each number) as activity counts and the activity levels were expressed as activity counts per epoch (epoch length is 15 seconds). To control the variability, each cat wore the same Actical through the entire period as far as this was possible.

Weight and body condition monitoring

The weight of the cats were monitored on day 1 and 5 of each week. In addition, the BCS (using the 7-point Waltham S.H.A.P. E^{TM} scale) was determined at the start of the study (week 1, day 1) and on the Friday afternoon at the end of the *ad libitum* test week (week 2, day 5).

Data processing and statistical analyses

Scale/data logger data was extracted from the device into Excel format. Each Excel file of the scale/data logger data was screened for feeding moments for which meal size (g), the number of

meals and the meal lengths (s) were recorded. Data were transferred into 1 Excel file and arranged per test day so every cat had 4 rows of data equaling 4 test days. A single meal was defined as 1 visit to the feeding station. Generally the meal length was calculated using the data logger data. When it wasn't clear from the data logger when a meal ended the camera footage was used to see when the cat had stopped eating. Other data included in that Excel file were body weight (BW), metabolic BW (BW^{0,75}), food intake in grams per kg of metabolic BW, energy requirement in kcal (see formula under Diets), total energy intake using the ME of the diets and the amount of food eaten, and the average meal time (total meal time divided by number of meals). Data was analysed using SPSS 22 (IBM SPSS Statistics Version 22). Student's t-tests were used to compare the 2 diets with each other in regard to intake, number of meals and meal duration. An analysis of variance (ANOVA) was used to compare the means of these different parameters on the 4 different test days for both diets with Day and Diet being fixed factors.

The Actical software analysed the Actical data and converted it to activity counts per epoch with an epoch length of 15 seconds. These activity counts from all cats were transferred to Microsoft Access where it was compiled into activity counts per clock hour per day for the whole test period. This was compiled into 4 groups into SPSS: Control, Control2, Diet HP, Diet LP. With control2 being the week after the test week. Actical count data was not normally distributed so negative binomial was used to account for the skewed distribution and the big spread. A generalized linear model was used to compare diet, day of week and clock hour for the variation in mean Actical count.

3. Results

Of 16 cats the meal patterns and activity counts were monitored. The feeding data of 2 cats were not used because of various problems during their test week (scale got unplugged, feeding station broke and needed repair). Two other cats did not have their test week because a sponsored study got priority and so this study was finished 2 weeks early. Twelve cats were therefore used for the meal pattern analysis. The 4 cats that were left out for meal pattern analysis did however give (partial) activity counts hence they are still part of the activity analysis.

Meal patterns

Combined meal pattern and intake data of the whole test week are presented in table 2. As shown in table 2, cats that are fed the HP diet have a significantly higher food intake than cats fed a LP diet (p=0.043) and because energy intake is transformed directly from the food intake, the daily energy intake with the HP diet is also significantly higher than with the LP diet. The daily eating time, number of meals taken per day and average eating time however are not significantly different from each other when comparing both diets. Also the energy intake/requirement ratio is not significantly different between diets.

Parameters	Palat	Palatability diet		
	High	High Low		
Eating duration (s/d)	2402±1081	2580±1310	0.614	
Nr. of meals (/d)	5.79 <u>+</u> 3.16	5.17 <u>+</u> 2.71	0.476	
Eating duration (s/meal)	505 <u>+</u> 250	566 <u>+</u> 341	0.490	
Food intake (g/d)	87.4 <u>+</u> 31.7	69.5 <u>+</u> 27.0	0.043	
Food intake (g/kg mBW/d)	39.56 <u>+</u> 14.1	31.73 <u>+</u> 11.8	0.045	
Energy intake (kcal ME/d)	327.5 <u>+</u> 119	260.5 <u>+</u> 101	0.044	
Energy intake/requirement	2.04 <u>+</u> 0.73	1.65 <u>+</u> 0.62	0.051	

Table 2: Mean meal pattern and intake values (\pm SD) of cats in general and fed either high palatable (n=6) or a low palatable (n=6) diet.

The effect of the test days on the meal pattern and intake when comparing diets is shown in the following tables.

First the mean number of meals per diet per day. Table 3 shows that there is a significant increase in number of meals taken as the test week progresses (P=0.013). However, when looking at the diet alone, there was no significant change in number of meals (P=0.476). And also the interaction between Day and Diet did not give a significant change in number of meals (P=0.879).

Day	Nr. of meals (/d)			
	High	Low		
Day 1	3.67 <u>+</u> 2.88	3.00 <u>+</u> 0.1.23		
Day 2	6.00 <u>+</u> 2.45	4.33 <u>+</u> 2.16		
Day 3	6.33 <u>+</u> 2.50	5.67 <u>+</u> 2.39		
Day 4	7.17 <u>+</u> 4.18	7.33 <u>+</u> 3.08		

Table 3: Mean number of meals $(\pm SD)$ per day for either the high-palatable of low-palatable diet. N = 12.

As can be seen in table 4 the metabolic intake stayed the same when taking the separate days into account (P=0.882). Diet alone did give a significant difference in food intake (P=0.045) in this case but the interaction of diet and day did not give a significant result (P=0.679).

Day	Food intake (g/kg mBW/d)			
	High	Low		
Day 1	36.79 <u>+</u> 16.74	29.44 <u>+</u> 16.55		
Day 2	42.48 <u>+</u> 19.31	27.93 <u>+</u> 11.13		
Day 3	41.85 <u>+</u> 9.39	32.94 <u>+</u> 8.08		
Day 4	37.15 <u>+</u> 11.73	36.23 <u>+</u> 12.61		

Table 4: Intake of food (g) per kg of metabolic BW $(\pm SD)$ per day for either the high-palatable or low-palatable diet. N = 12.

In table 5 the energy intake versus energy requirement ratio is shown. If the ratio is higher than 1 the cats had a higher energy intake than they required. As seen in the table, on all days the ratio>1. When looking at the individual ratios per day (data not shown), only on a few rare occasions did the ratio drop below 1, the average for each cat however stayed above 1. There is a definite trend when looking at the differences of the ratio between the 2 diets (P=0.051), but again nothing when looking at the day (P=0.894) and the interaction of day and diet (P=0.671).

Day	Energy intake/requirement			
	High Low			
Day 1	1.90 <u>+</u> 0.85	1.54 <u>+</u> 0.88		
Day 2	2.20 <u>+</u> 0.99	1.45 <u>+</u> 0.57		
Day 3 2.16±0.50		1.71 <u>+</u> 0.41		
Day 4	1.92 <u>+</u> 0.60	1.88 <u>+</u> 0.65		

Table 5: Ratio of energy intake versus energyrequirement per day (\pm SD) for either the high-palatable or low-palatable diet. N = 12.

Table 6 shows the total feeding duration per day and the duration of an average meal per day. The table shows that there is no significant effect of either day or diet on daily eating duration (P=0.290 and P=0.679) nor meal duration (P=0.323 and P=0.481) respectively.

Day	Eating dura	ation (s/d)	Eating durat	ion (s/meal)
	High	Low	High	Low
Day 1	2187 <u>+</u> 1575	1828 <u>+</u> 1189	711 <u>+</u> 315	617 <u>+</u> 376
Day 2	2410 <u>+</u> 1155	2143 <u>+</u> 1445	452 <u>+</u> 242	521 <u>+</u> 239
Day 3	2717 <u>+</u> 845	2997 <u>+</u> 1209	471 <u>+</u> 172	645 <u>+</u> 480
Day 4	2297 <u>+</u> 809	3227 <u>+</u> 1167	389 <u>+</u> 170	491 <u>+</u> 301

Table 6: Total feeding time $(\pm SD)$ and average meal duration $(\pm SD)$ per day for either the high-palatable or low-palatable diet. N= 12.

As stated before, cats fed the HP diet had a significantly higher food intake compared to LP fed cats. While not significant, results do give the impression that LP fed cats have a longer feeding duration, but less meals throughout the test week. Also the average meal duration is longer than in HP fed cats. This could imply that cats fed the LP diet have a slower eating rate compared to HP fed cats. Data collection was not specific enough to calculate an accurate eating rate.

Activity

Actical data were collected from all 16 cats. Not all cats wore their Actical collar for the whole test period due to various reasons. One Actical gave corrupted data from 2 separate weeks so the data of those 2 weeks was lost (1 control and 1 HP diet week). Because the study had to end prematurely, the second control week of 2 other cats was not measured and the test week and second control week of yet 2 other cats was not measured. On a few occasions, the Actical collars came loose from the cats' necks. Consequently, cats started to play with the Actical devices lying on the floor which was the reason for several extreme activity values. Therefore a cut-off point of 95% was instituted to account for these extremes. The 5% highest values were not used for statistical analyses.

Figure 1 shows the mean Actical count throughout a 24 hour period over the 3 week measuring period. As can be clearly seen in the figure, the activity counts rise around 07:00 hour, and again at 15:00 hour, as these are the times the cats are normally being fed.



Fig 1: Mean daily activity pattern (activity counts/epoch) of all cats (n=16) per clock hour.

Table 7 shows the average daily activity for the 4 different weeks in activity counts/epoch. The average activity in the HP diet week is significantly lower when compared with control week 1 (P=0.01). Furthermore, the average activity in the LP diet week is significantly lower than the average in control week 1 (P<0.001) and 2 (P=0.003). There was no significant difference found between both control weeks (P=0.25), nor between both Diet weeks (P=0.33).

Week	Activity (activity counts/epoch)		
Control 1	3949 <u>+</u> 82.34		
Control 2	3814 <u>+</u> 82.32		
HP Diet	3536 <u>+</u> 137.12		
LP Diet	3364 <u>+</u> 123.74		

Table 7: Average daily activity $(\pm SE)$ of the 2 control weeksand the 2 test weeks.

As can be seen in Figure 2, the different groups still had a similar activity pattern with high activity around meal times (07:00 hour and 15:00 hour). The HP diet cats (n=6) showed a significantly lower activity (P<0.05) around 15:00 hour but not around 07:00 hour from the 3 other groups. Around 11:00 hour however, the activity count in the HP group was significantly higher (P<0.05) compared to the other groups. Significant results were found when the difference between two means was greater than twice the maximum standard error of the difference. An interaction model was used to test the effects of week (control, control2, Diet HP and Diet LP), clock hour and day of week on the mean activity levels. All fixed effects had a significant interaction on activity counts, with the P-value being 0.001, <0.001 and <0.001 respectively. However, when weekend days were taken out of the equation, the effect day of week did not show a significant interaction (P=0.5) on mean activity counts.



Fig 2: Mean daily activity pattern (actical counts/epoch) of cats in different weeks of the study per clock hour. Control n = 15, Control2 n = 12, Diet HP n = 6, Diet LP n = 7.

4. Discussion

The current study was used to evaluate the effect of a high palatable diet versus a low palatable diet, both given on an *ad libitum* basis, on the physical activity, bodyweight and meal patterns of 16 cats. The studied meal patterns included number of meals, meal duration and quantity of food/energy intake. Finding differences between 2 diets both given *ad libitum* might give clues about possible strategies to counter the obesity problem.

Cats fed the HP diet had a significantly higher food intake compared to cats fed the LP diet (P=0,045). In a previous study, Mengdie Cao [2014] had already found that the HP diet was more desirable than the LP diet in a preference test. Studies in humans [Yeomans et al., 1997; Erlanson-Albertsson, 2005] also showed that palatable food is responsible for a higher food intake compared to bland food. However this influence of palatability does not need to be absolute, as Sunday et al. [1983] showed in their study with rats. When offered 2 nutritionally equivalent diets simultaneously, the palatable diet was eaten faster, in larger amounts and more frequently than the less palatable diet, which was expected. When the 2 diets were offered sequentially however, there was no difference in number of meals, feeding time or feeding rate. This suggests that palatability only affects meal pattern parameters when there is an option to choose between 2 or more diets. When diets are presented sequentially the palatability influence is not strong enough to be responsible for a change in meal pattern parameters. Whether this effect also occurs in cats is unknown as there are no studies with cats with this particular experiment design as far as could be found. The meal pattern parameters of the cats' control diet were not tested in this study. Therefore it is not possible to compare the 2 test diets with the control diet. It would have been interesting to see though if the control diet was comparable to the HP or the LP diet when looking at meal pattern parameters. While the test diets were specifically made to be palatable and less palatable one can argue that the control diet is already a very palatable diet as it has a large amount of protein and only a little less fat compared to the test diets. And both fat and animal protein are key factors in the palatability of feline diets [Hand et al., 2010]. Sunday et al. [1983] also found in their study that meal pattern parameters were not increased with the more palatable diet but rather reduced with the less palatable diet. With the present study it was not possible to observe if this was also the case in cats.

The number of meals taken became significantly higher as the week progressed. There was however no difference between the HP and LP group. It was expected that the number of meals would rise to between 7 and 20 meals per day. Several studies showed that, when given a free food-choice, cats consume 7 to 20 meals per day to ingest their daily energy need [Kanarek, 1975; Kaufman et al., 1980; Kane et al., 1981b; Bradshaw et al., 1996]. As already mentioned, there was a significant increase in the number of meals that were consumed when comparing the first day to the fourth day. The number of meals rose from 3.67 and 3.00 to 7.17 and 7.33 meals per day for the HP and LP diets respectively. As Mugford et al. [1977] previously concluded, there is a big variation between individual cats. Which was also apparent in the present study with some cats eating 3-4 meals on the last test day and others eating 14 meals. Even though some cats consumed the expected number of meals per day, the energy intake of these cats far exceeded their normal daily energy need. A possible explanation for this overeating could be that the cats are accustomed to 2 relative small meals a day, maybe even experience a bit of hunger now and then. The overall low BCS and

bodyweight (Appendix 1) could indicate the same. When food is normally scarce, but is abundant all of a sudden due to the *ad libitum* access to food in this study, the cats lose control and eat much more than they need to. If the duration of the study would have been longer it is very likely that we would have seen an increase in body weight as was seen in a previous study [Goggin et al., 1993]. Goggin et al. [1993] placed six cats from a body-weight maintenance diet on an *ad libitum* diet for 80 days. Every cat showed a high caloric intake for a long time. It took almost 80 days for the cats to adapt to this new feeding method to once again reach their maintenance caloric intake. During this time 67% of the cats had become obese. In the current study, there was no sign of adaptation to the new diet in relation to caloric intake as the intake/requirement ratio was too high and stayed the same throughout the week. The 3 occasions before the test week and the Monday of the test week itself were not enough to let the cats adapt to a new *ad libitum* feeding regime. For a better preparation for future research an appropriate adaptation period is needed before the actual test period. The adaptation period is good enough when the animals reach a stable bodyweight again. When an animal needs to be adapted to a new diet via meal feeding, one can simply use the maintenance requirement of the animal, as calculated from a few weeks of weight-consistency [Wichert et al., 2012]. When planning on a study with a new *ad libitum* feeding regimen, the adaptation period is as long as the animals need to reach stable bodyweights by themselves. This usually takes much longer as Goggin et al. [1993] needed almost 80 days and Wei et al. [2011] for 3-4 months.

The energy intake/energy requirement ratio showed no significant result for either diet (P=0.051) or day (P=0.894). One can however, speak of a clear trend for the type of diet given. The trend showed that cats fed the HP diet had a larger ratio (2.04) compared to the LP diet (1.65). This means that cats fed the HP diet ate, on average, more than twice their daily caloric need and LP diet cats more than 1.5 times their daily need, assuming the ratio was 1 before the study started. Before this study, the cats were fed so they would maintain their constant, lean BCS scores. For this study however, the weight of the cats at the start of their test week was used to base their caloric need on. This calculated need could deviate from the original maintenance need which could be a reason for a less accurate portrayal of the actual intake/requirement ratio. But it is unlikely that this would give a significantly different result as the ratios for both diets are quite high.

Both total feeding time and average meal time were not significantly different between the HP and LP groups. However, one cannot help but notice when looking at Table 6, that it seems that, as the week progressed, the eating duration per meal decreased for both diets. But because of large individual differences ranging from 117s per meal to 1020s per meal this was not significant at all. It is probable that with a suitable adaptation period and/or a quieter environment, these huge individual differences could be negated. The cats learned very quickly to operate the feeding station effectively. However, the training was done individually. When in their facilities the more submissive cats in the group were often pushed back by the dominant cats when trying to enter or exit the feeding station. This did not give a positive reinforcement for the submissive cats to take numerous small meals but rather reinforce the behaviour of eating larger meals less frequently to avoid confrontations. A solution for this problem could be to reduce the amount of cats present in the room. A suitable adaptation period beforehand would not help in this case as the submissive cats would still be compliant toward the dominant cats.

The total feeding time per day is probably more dependent on the palatability of the diets again. While not significant, the apparent difference in total eating duration in the LP group, as the week progressed, is notable. The reason behind the apparent increase in eating duration is most likely due to reluctance to eat the less palatable food. As Mengdie Cao [2014] proved in her two-bowl preference test where she not only looked purely at the palatability of both diets but also at the feeding rate at which both diets were consumed. She observed that the HP diet was consumed significantly faster (and in higher quantities) than the LP diet (P<0.001). Which further emphasizes the preference for the HP diet and shows that the LP diet is consumed in a slower rate.

Weight and body condition score of the cats did not change at all during the study. It was expected that the *ad libitum* fed cats would eat large quantities of the food which in turn would result in weight gain, as was shown by Van der Weijden [2014] in a previous study. Large quantities of food were eaten, but no weight gain was observed. As mentioned in the introduction, researchers cannot agree about whether or not *ad libitum* feeding is directly responsible for overweight in cats or if it could even help cats stay lean. As of yet there is no definite consensus. The recent survey of Van der Weijden showed that only 14 out of 147 household cats that were fed on a *ad libitum* basis were considered overweight (9,5%). Whereas 147 out of 585 household cats that were meal fed were considered overweight (25%). The conclusion of the survey was therefore that *ad libitum* feeding the optimal method was to keep your cat in good shape. But as mentioned before, there are as many studies that state that *ad libitum* feeding has a positive effect on weight management, as there are studies that contradict that conclusion. Despite all this, a more probable reason why no weight gain was observed in this instance was probably due to the short test period.

Average physical activity of both the HP (P=0.01) and LP (P<0.001) diet week is significantly lower when compared to control week 1. Additionally, the average activity of the LP diet week (P=0.003) is also significantly lower when compared to control week 2. Deng et al. [2011; 2014] concluded that a higher quantity of meals resulted in a higher physical activity. But only when cats were meal fed. The effect of ad libitum feeding on physical activity was not researched. The reason behind this is because of food anticipatory activity (FAA). This FAA only occurs when animals are being fed at scheduled times every day. The cats learn that they get food every day around the same time and just before the scheduled time their activity increases in anticipation of a meal. Deng et al. [2014] were the first to demonstrate the occurrence of FAA in domestic cats using quantitative measures. Previous studies already demonstrated it in rats [Mendoza et al., 2005], mink [Vinke et al., 2004] and horses [Peters et al., 2012]. In the present study, all cats were used to being fed twice daily and thus had FAA right before meal times around 07:00 and 15:00 hour (as can be clearly seen in both figures). The cats that are given an *ad libitum* diet should theoretically learn that there is no point in spending energy in anticipation of a meal because food is always accessible to them. This should make their mean activity lower during their test week. But because the cats were still housed all together, the FAA of the cats with the normal feeding routine still influenced the activity pattern of the cats in their test week. Therefore the same activity spikes are seen around 07:00 and 15:00 hour in figure 2 for the test weeks, only the HP diet group showed a significant decrease in activity at the second spike. A remarkable activity spike is present in Figure 2 at around 11:00 hour. The HP Diet group suddenly had a significantly higher activity count compared to the other 3 groups. The most plausible explanation for this abnormality is that one of the Acticals came loose from a cat and spiked a huge activity count that stayed just below the cut-off value. To test whether or not the activity of the cats is influenced by which week it participates in, which day it is or what the hour of the day is, a

interaction model was used. All three models had a significant influence on the activity count. The significant effect (P=0.001) of the type of week is already discussed above. The day of the week also has a significant effect (P<0.001) on the activity count. When looking at the pair wise comparisons of the different days it becomes clear that the significance of this factor is due to the significant contrasts of the Saturday and Sunday. The activity of the cats on Saturday and Sunday is very low compared to normal weekdays. Apparently the cats react strongly on the presence of people and calm down when no one is around. The hour of the day also had a strong significant (P<0.001) influence on activity which is not surprising when looking at the huge activity spikes in Figure 2.

To improve a possible subsequent study, 2 issues should be addressed: the lack of an adaptation period and the influence of the meal fed cats. The adaptation period for the *ad libitum* test week would have ensured that the cats were already used to the fact that food is always available. This would give a better image of the actual activity pattern of *ad libitum* fed cats. The current result was still mostly overshadowed by the FAA. Which is part of the second issue, the influence of the meal fed cats. The meal fed cats get fed twice a day at the same times. Right before feeding time the FAA of the other cats is enormous. Because of the group housing, the *ad libitum* fed cats gets excited as well and as a result will show a different activity pattern than it would normally have done if they would have been alone or together with other *ad libitum* fed cats. As long as the *ad libitum* fed cats are housed in the same room as the meal fed cats, the FAA will probably keep influencing the activity pattern of the cats in the test period.

5. Conclusion

The main question of the study was what the effects would be of an *ad libitum* feeding method with either a high- or low-palatable diet, on physical activity, bodyweight and on the feeding patterns of domestic cats. When cats in the wild come into contact with an *ad libitum* food situation, they can adapt to it in the way that they will eat frequently but only small amounts of food each time. However, most cats in the current study did not seem to adapt during the test period. Both diets were eaten more frequently as the test period progressed. However, the amount of food eaten by both groups also increased. The HP diet cats at e significantly more than the LP diet cats, even as the LP diet cats ate too much already. This resulted in a large energy intake that exceeded the energy requirement of the majority of the cats. However, no changes at all were observed concerning bodyweight and BCS. Probably because the test period of 4 days was too short. If the test period would have been longer however, it is very likely that an increase in bodyweight would have occurred. The other feeding pattern parameters showed possible trends but due to large variations between individuals the results were not significant. The only effect of *ad libitum* feeding on the physical activity is that both diet groups got a lower activity count when compared with the control week. That can be explained by the lessened food anticipatory activity (FAA).

With the results of the current study it is not possible to formulate a satisfactory answer to the research question. However, there are some suggestions that could perhaps improve the study design for a possible subsequent study. The most important study design improvement would be to extend the adaptation period before the actual test period. The adaptation period would be used to prevent the overeating problem as the cats would have had a long enough time before the actual test period to get used to the new diet. The duration of the period will be as long as it takes for the cats to stabilize their weight on the new diet. In addition, the adaptation period would be useful for activity measurements of the cats in their *ad libitum* test week to make sure they know that food is always available so the FAA is as low as possible. With perhaps a change in housing to decrease the influence of meal fed cats. Another improvement would be a longer study duration, especially when bodyweight and BCS are important as they do not change significantly that fast.

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Zaghini, G. and G. Biagi (2005), Nutritional peculiarities and diet palatability in the cat, Vet. Res. Commun., 29(SUPPL. 2), 39-44. **INDEX 1:** Table which shows basic stats of each cat that participated in the study. BCS: Body Condition Score at the start of the study period; Waltham S.H.A.P.E. (7-point scale A to G) scored on 19-05-2014. Diet: LP = low-palatable diet, HP = high-palatable diet. BW: Bodyweight (in kg) at the start of test week. ER: Energy requirement in kcal based on metabolic BW.

Cat name	Gender	Birth date	BCS	Diet	BW	ER
Karine	9	26-12-2011	D	LP	2.74	156.49
Anouk	9	06-05-2012	D	HP	2.78	158.89
Aal	9	01-04-2012	D	LP	2.79	159.50
Carolien	9	01-04-2012	С	HP	2.67	151.93
Bone	9	26-12-2011	В	LP	2.31	130.55
Bella	9	26-12-2011	С	HP	2.82	161.32
Oor	Ŷ	20-05-2012	В	LP	2.43	137.75
Jill	9	07-05-2012	С	HP	2.69	153.44
Jacob	8	01-04-2012	В	LP	3.28	175.97
Jack	8	21-04-2012	С	HP	3.11	165.83
Panter	8	06-05-2012	В	LP	3.03	161.08
Absint	8	04-12-2011	В	HP	3.15	168.21
Edward	2	01-05-2012	С	LP	3.42	184.37
Skye	2	20-02-2012	В	HP	3.45	186.17