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Effect of sterilization and of dietary fat and carbohydrate content on food intake, activity level, and blood satiety–related hormones in female dogs[1]

S. Schauf,* A. Salas-Mani,† C. Torre,† G. Bosch,‡ H. Swarts,§ and C. Castrillo*[2]

*Departamento de Producción Animal y Ciencia de los Alimentos, Universidad de Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain; †Affinity Petcare, Research and Development Department, 08174 Barcelona, Spain; and ‡Animal Nutrition Group and §Human and Animal Physiology Group, Department of Animal Sciences, Wageningen University, PO Box 338, 6700, Wageningen, the Netherlands

ABSTRACT: Animal sterilization is suggested to promote food overconsumption, although it is unknown whether this effect is mediated by variations in satiety-related hormones, which are released in response to food intake. The aim of this study was to evaluate the effect of sterilization and of the main energy-delivery nutrients, fat and nonstructural carbohydrates, on food intake, blood concentration of satiety-related hormones, and activity level in dogs. In a 2-phase experiment (phase I [Ph.I], 74 d, and Ph.II, 84 d), 12 female Beagle dogs were assigned to a control group (intact in both phases) and a sterilization group (spayed 20 d before Ph.II). In each phase, dogs received a high-carbohydrate (HC) diet (313 and 105 g/kg DM starch and fat, respectively) and a high-fat (HF) diet (191 and 213 g/kg DM starch and fat, respectively), both high in total dietary fiber (>200 g/kg DM) and providing 27% ME as protein, in 2 consecutive periods following a crossover arrangement. During each period, dogs' voluntary DMI and activity level were recorded during 5 d. Then, energy allowance was restricted to 0.7 maintenance and the level of intake of a common challenge food offered 4 h after feeding the experimental diets (challenge food intake [ChFI]) was used as an index of the satiety state of dogs. Blood concentration of active ghrelin, cholecystokinin (CCK), total peptide YY (PYY), and insulin were determined before and 15, 60, 120, 240, and 360 min after feeding. Voluntary DMI was greater ($P < 0.05$) in HF-fed dogs, but ChFI did not differ between diets ($P > 0.10$). Dogs fed the HF diet showed a lower increase of CCK at 120 ($P < 0.01$) and 240 min ($P < 0.05$), resulting in a lower ($P < 0.001$) total area under the curve from 0 to 240 min ($tAUC_{0–240}$). A lower PYY elevation ($P < 0.05$) was also found in HF-fed dogs at 120 min. Only active ghrelin concentration at 240 min and insulin $tAUC_{0–240}$ correlated ($P < 0.05$) with ChFI ($r = 0.357$ and $r = −0.364$, respectively), suggesting a role of these hormones in appetite. Dog sterilization did not affect voluntary DMI, ChFI, or blood hormones ($P > 0.10$) but led to a reduced activity level compared with control dogs ($P < 0.05$). In summary, dog sterilization was not associated with an impaired appetite control. Feeding dogs the HF diet led to energy overconsumption and to a lower blood elevation of CCK and PYY but was not associated with a weaker satiating effect 4 h later compared with the HC diet.

Key words: activity, dietary fat and carbohydrates, dog sterilization, food intake, satiety hormones.

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INTRODUCTION

Food intake control involves the secretion of gut-derived hormones, which exert an orexigenic (ghrelin) or anorexigenic (i.e., cholecystokinin [**CCK**], total peptide YY [**PYY**], and insulin) action in a meal-to-meal context (Hameed et al., 2009), with the response differing between dietary macronutrients (Karhunen

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[2]Corresponding author: ccastri@unizar.es

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et al., 2008). Conflicting results exist regarding carbohydrates and fat, with a series of human studies reporting a weaker effect of fat on satiety (Blundell et al., 1993; Lawton et al., 1993), whereas in dogs, Geoghegan et al. (1997) found a larger suppression of sham feeding following an intraduodenal infusion of oleate compared with an intraduodenal infusion of dextrose. Carbohydrates are regarded most effective in suppressing ghrelin and in increasing insulin after a meal in humans, whereas fat is found to elicit a major increase of CCK and PYY (Karhunen et al., 2008). Available data in dogs is limited, with Greeley et al. (1989) reporting a major increase of PYY after intraduodenal fat and Lubbs et al. (2010) reporting a similar ghrelin response after oral loads of fat and carbohydrates, and a relationship between blood hormone variations and food intake is not consistently established (Bosch et al., 2009). On the other hand, there is evidence that estradiol attenuates the orexigenic effect of ghrelin (Clegg et al., 2007) and enhances the satiating potency of CCK (Butera et al., 1993) and PYY (Papadimitriou et al., 2007). Accordingly, an increase of food intake has been reported in dogs after sterilization (Houpt et al., 1979; Jeusette et al., 2004), although hitherto, its effect on gut hormones remains undetermined. The objective of this study was to evaluate the effect of feeding high-carbohydrate or high-fat diets on food intake and blood concentration of gut-derived hormones in dogs before and after sterilization. We hypothesized that dog sterilization would result in food overconsumption via variations in gut hormones and that this response would be differentially evoked by fat and carbohydrates.

MATERIALS AND METHODS

Animal housing and experimental procedures were approved by the Ethic Committee for Animal Experimentation of the University of Zaragoza (PI26/13).

Animals and Diets

Twelve intact female Beagle dogs aged between 2 and 6 yr with a mean BW of 13.6 ± 0.6 kg and BCS of 5.3 ± 0.3 on a 9-point scale (optimal 5/9; Laflamme, 1997) were randomly assigned to a sterilization group (**SG;** $n = 6$) and a control group (**CG**; $n = 6$) according to their BW and age. The dogs were individually housed in indoor concrete floor kennels (2.0 by 2.5 m) with free access to outdoor courts (2.0 by 5.0 m). Indoor temperature was maintained between 18 and 24°C throughout the experiment and drinking water was provided ad libitum. Two dry extruded diets were formulated to meet the Association of American Feed Control Officials (AAFCO, 2011) nutritional recommendations. Under these premises, a diet high in potentially digestible carbohydrates, in terms of nitrogen-free extractives (**NFE**), and low in fat (high carbohydrate [**HC**]) and a diet high in fat and low in NFE (high fat [**HF**]), both with a high content in total dietary fiber (>200 g/kg DM), were formulated. Both diets had a similar CP content on a ME basis (approximately 27%) to procure a similar level of protein intake in g/kg $BW^{0.75}$ when offered at the same level of intake (in kJ/kg $BW^{0.75}$). Ingredient and chemical composition of the experimental diets is shown in Table 1. The ME content of both diets was estimated using the NRC (2006) approach as the product of deter-

Table 1. Ingredient and chemical composition of the experimental diets

	Diets[1]	
Item	HC	HF
Ingredient composition, g/kg, as-fed basis		
Corn	254.4	20.0
Poultry byproduct meal (63% CP and 17% ash)	202.8	204.1
Corn gluten meal (60% CP)	79.9	136.4
Lard	24.4	123.0
Rice	93.9	95.4
Barley	75.1	95.4
Soybean meal (48% CP)	45.1	95.4
Pea fiber	56.3	57.2
Hydrolyzed pork liver	50.7	51.5
Cellulose	40.4	41.0
Beet pulp	37.6	38.1
Vitamin–mineral premix[2]	31.0	33.0
Gum arabic	4.7	4.8
Guar gum	4.7	4.8
Analyzed chemical composition, g/kg DM		
Ash	77.5	78.7
CP	269.0	307.0
Ether extract	105.0	213.0
Crude fiber	82.7	78.9
Nitrogen-free extractives[3]	465.8	322.4
Starch	313.0	191.0
Total dietary fiber	226.0	211.0
Insoluble dietary fiber	198.0	174.0
Soluble dietary fiber	28.2	37.3
Energy content, MJ/kg DM		
GE[4]	20.0	22.8
ME[5]	14.8	16.9

[1]HC = high-carbohydrate; HF = high-fat.

[2]Supplying, per kilogram diet, 3.19 g Na, 5.96 g Cl, 1.77 g K, 0.280 g Mg, 200 mg Fe, 7.14 mg Cu, 198 mg Zn, 50.5 mg Mn, 0.23 mg Se, 0.146 mg Co, 7.29 mg vitamin A, 0.040 mg vitamin D_3, 446 mg vitamin E, 0.410 mg vitamin K_3, 41.4 mg vitamin B_1, 20.2 mg vitamin B_2, 150 mg vitamin B_3, 38.2 mg vitamin B_5, 10.6 mg vitamin B_6, 0.080 mg vitamin B_7, 5.07 mg vitamin B_9, 0.120 mg vitamin B_12 and 315 mg vitamin C.

[3]Calculated by subtracting the ash, CP, ether extract, and crude fiber contents from the DM.

[4]Determined by calorimetric bomb.

[5]Estimated as GE × GE digestibility coefficient (91.2 − [1.43 × % crude fiber]/100) and assuming urinary energy losses of 4.35 kJ/g CP (NRC, 2006).

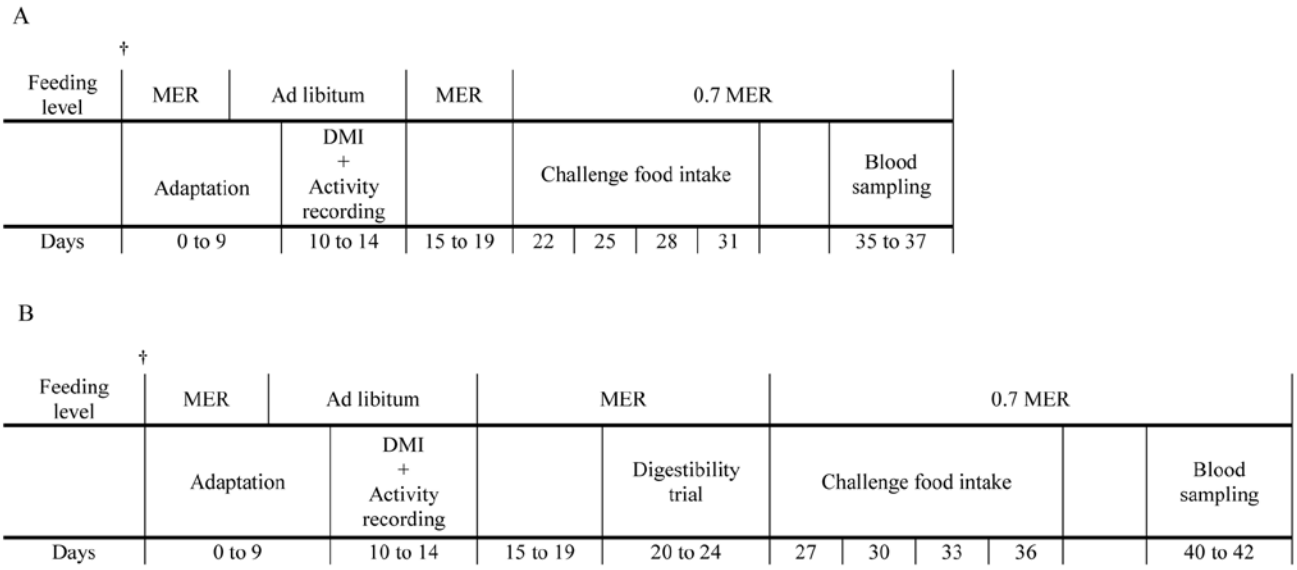

Figure 1. Experimental procedures followed in each of the 2 crossover periods of phase I (A) and phase II (B) of the study. In each figure, the timing of BW record (*), voluntary DMI, activity recording, challenge food intake, digestibility trial, and blood sampling is shown. MER = maintenance energy requirements.

mined GE multiplied by the apparent GE digestibility coefficient (GE digestibility = [91.2 − (1.43 × % crude fiber, in DM)/100]) and assuming urinary energy losses of 4.35 kJ/g CP. This estimated ME content was used to adjust the level of intake throughout experiment.

Experimental Design and Procedures

The study lasted 6 mo and comprised 2 phases (phase I [**Ph.I**] and phase II [**Ph.II**]). In each phase, all of the dogs were fed the HC and HF diets in 2 consecutive periods following a crossover arrangement. The time schedule of procedures followed in each of the 2 periods of Ph.I and Ph.II is shown in Fig. 1A and 1B, respectively. Phase I started at the beginning of the summer and lasted 74 d (37 d/period), during which all of the dogs were intact. Afterward, dogs of the SG were spayed in the Veterinary Hospital of the University of Zaragoza (Zaragoza, Spain), and, after a 20-d recovery period, Ph.II was conducted for 84 d (42 d/period). Each period in Ph.I consisted of 5 d of adaptation to the diets, which were individually fed at 0900 h to meet the estimated maintenance energy requirements (**MER**) for laboratory Beagle dogs (552 kJ ME/kg optimal $BW^{0.75}$·d; NRC, 2006). The optimal BW of dogs was estimated at the beginning of the study by increasing or reducing the starting BW 10% per unit of BCS below or above 5 (German et al., 2009). Food allowance was gradually increased in the following 4 d until food refusals were ensured, and from 10 to 14 d food refusals were collected daily, pooled on an individual basis, and dried to calculate the average daily voluntary DMI. Afterward, dogs were adapted to consume their daily food from 0900 to 0920 h at a level of intake close to MER for 5 d and at 0.7 MER for the next 18 d. This level of energy restriction matches the estimated daily energy intake required for the maintenance of BW in spayed dogs (Jeusette et al., 2004). After 2 d at 0.7 MER, 4 satiety tests were conducted leaving 2 d in between consecutive tests. In each satiety test, dogs were offered a challenge food 240 min after consumption of the experimental diets. The amount of challenge food offered (600 g, as-fed basis) was based on the level of food allowances that assured food refusals during ad libitum feeding. The challenge food was a commercial dry extruded diet (Brekkies Excel; Affinity Petcare, Barcelona, Spain) providing 230 g CP, 140 g ether extract (**EE**), and 2.70 g crude fiber (**CF**) per kilogram food, on an as-fed basis, as specified in the label, with an estimated ME content of 14.9 MJ/kg fresh matter, by applying the Atwater factors 14.63, 35.56, and 14.64 kJ per gram of CP, EE, and NFE, respectively. After 20 min, food refusals were collected and the challenge food intake (**ChFI**) of each dog was recorded.

Physical activity was assessed using activity monitors (Actical Philips Respironics, Amsterdam, the Netherlands) attached to the collar of 5 dogs from each group (CG and SG) during ad libitum feeding. The device consisted of an omnidirectional accelerometer that compiled the intensity and duration of the movements and converted them into arbitrary numbers referred to as activity counts (Hansen et al., 2007). Accelerometer data epoch was set at 1 min and daily activity was expressed as average counts per minute.

On the last 3 d of each period, blood samples were collected before meal distribution (time 0) and at 15, 60, 120, 240, and 360 min after consumption of the experimental diets at 0.7 MER (2 dogs per diet/d). The day before blood sampling, dogs were sedated using 0.20 mg/kg BW of acepromazine (Calmo Neosan; Pfizer, Madrid, Spain) and 0.01 mg/kg of buprenorphine (Buprex; Schering-Plough, Madrid, Spain), and a venous catheter (BD Instye-W 18 G, 1.3 × 45 mm; Becton Dickinson, Madrid, Spain) was placed in the jugular vein. Catheter patency was maintained by flushing with 1 mL heparinized saline (10 IU of heparine/mL 0.9% NaCl solution) at the time of catheter placement and before and after blood sampling (approximately 0.5 mL). Blood flushed with the heparinized solution was discarded before sampling.

Samples for active ghrelin, CCK, and PYY (2 mL blood per hormone) were collected in chilled tubes containing 1.8 mg K3 EDTA per 1 mL blood (Aquisel, Barcelona, Spain). Tubes for CCK and PYY analyses contained 10 μL/mL blood of an aprotinin solution (10 mg aprotinin bovine [Sigma-Aldrich Chemie GmbH, Steinhelm, Germany] dissolved in 1 mL of 0.9% NaCl solution). After gently mixing, tubes were kept on ice until centrifugation (within 30 min after blood collection). For insulin analysis, 2 mL of blood were collected in serum separator tubes (BD Vacutainer; Beckton Dickinson, Plymouth, UK) and allowed to clot at room temperature. All tubes were centrifuged at 2,000 × *g* for 10 min at 4°C. For active ghrelin analysis, plasma samples were transferred to safe-lock tubes (Eppendorf, Hamburg, Germany) containing 10 μL of a phenylmethylsulfonyl fluoride solution (10 mg phenylmethylsulfonyl fluoride [Sigma-Aldrich Chemie GmbH] dissolved in 1 mL 100% isopropanol) and 50 μL of HCl (1 N) per 1 mL of plasma. All samples were stored in cryovials at −80°C until analyses.

In Ph.II, both periods included 9 d more between ad libitum feeding and the satiety tests, during which the apparent digestibility of the experimental diets was determined in both spayed and intact dogs. Briefly, dogs were fed the experimental diets at a level of intake close to the MER for 5 d and then food intake and feces were recorded daily during the next 5 d. Feces from each dog were collected directly from the kennel floor twice a day, dried at 65°C for 48 h, pooled per dog at the end of the collection period, and stored until analyses. Dogs were weighed at the beginning and at the end of each experimental period in Ph.I and Ph.II.

Analytical Methods

Samples of the HC and HF diets were collected weekly and pooled per phase. Food and dried fecal samples were milled to pass a 1-mm screen and analyzed for DM and ash contents by drying to a constant weight at 105°C and combustion at 550°C for 6 h, respectively. Analyses of food and fecal CP, EE, and CF were performed according to AOAC (2005) procedures (methods 976.05, 954.02, and 978.10, respectively). The NFE content of the diets was calculated by subtracting the ash, CP, EE, and CF from the DM. The total dietary fiber content of foods was calculated as the sum of soluble and insoluble dietary fiber, according to methods 993.19 and 991.42, respectively, outlined by the AOAC (1995). The starch content of the diets was enzymatically analyzed (K-TSTA kit; Megazyme International, Wicklow, Ireland). D-Glucose and maltodextrins were previously washed with 80% ethanol and resistant starch was predissolved by stirring the sample with 2 *M* KOH at 4°C. The GE content of food and feces was determined in an adiabatic bomb calorimeter (IKA C-4000; Janke-Kunkel, Staufen, Germany). The ME content of the diets was calculated by subtracting 5.23 kJ/g digestible CP from their DE (NRC, 2006). Commercial RIA kits from Linco Research (Billerica, MA; 100% cross-reactivity with canine ghrelin, PYY, and insulin), previously applied in dogs (Bosch et al., 2009; Zietlow et al., 2010), were used for the determination of blood insulin (human-specific insulin RIA with a detection limit of 2.715 microunits/mL and intra-assay and interassay CV of 3.3 and 4.5%, respectively), total PYY (rat–mouse PYY RIA with a detection limit of 78.1 pg/mL and intra-assay and interassay CV of 3.6 and 8.5%, respectively), and active ghrelin (Ghrelin active RIA, with a detection limit of 7.8 pg/mL and intra-assay and interassay CV of 7.4 and 13.5%, respectively). Plasma CCK was determined using a RIA procedure developed to bind to different bioactive forms of CCK in mammals (CCK-8, CCK-22, CCK-33, and CCK-58). The detection limit was 0.1 p*M* and the intra-assay and interassay CV ranged from 4 to 15% (Rehfeld, 1998).

Calculations and Statistical Analyses

All data were analyzed using the Statistical Analysis Systems software package version 9.2 (SAS Inst. Inc., Cary, NC). For active ghrelin, CCK, PYY, and insulin, the sum of the net (baseline subtracted) area under the curve (**AUC**) from 0 to 120 min (**$nAUC_{0–120}$**) and from 120 to 360 min (**$nAUC_{120–360}$**) and the net and total AUC from 0 to 240 min (**$nAUC_{0–240}$** and **$tAUC_{0–240}$**, respectively) after feeding the experimental diets were approximated using the trapezoidal summation method ($\sum$area = $\sum h_i \times b_i$, in which *h*i is the mean score of pairs of adjacent time points and *b*i is the time interval between these points) and weighted by time. In Ph.II, the effects of diet and of sterilization (group effect) on nutrient and GE digestibility were analyzed using the mixed

Table 2. Effect of diet and sterilization (group effect) on nutrient and energy apparent digestibility coefficients and on the energy content of the experimental diets[1]

	Diet[2]		Group[3]				*P*-value[6]	
Item	HC	HF	CG	SG	SED[4]	SED[5]	Diet	Group
Digestibility								
DM	0.726	0.740	0.731	0.735	0.007	0.013	0.087	0.744
OM	0.754	0.767	0.758	0.763	0.006	0.012	0.070	0.728
CP	0.814	0.841	0.827	0.828	0.006	0.007	0.002	0.892
Ether extract	0.857	0.921	0.888	0.891	0.004	0.010	<0.001	0.734
Crude fiber	0.182	0.125	0.119	0.186	0.042	0.044	0.212	0.159
GE	0.764	0.803	0.781	0.786	0.006	0.018	<0.001	0.355
Energy content								
DE, MJ/kg DM	15.3	18.3	16.7	16.8	0.1	0.2	<0.001	0.355
ME,[7] MJ/kg DM	14.2	16.9	15.5	15.6	0.1	0.2	<0.001	0.358

[1]Values are least squares means for each diet (n = 12) and each animal group (n = 12).

[2]HC = high-carbohydrate; HF = high-fat.

[3]CG = control group; SG = sterilization group. Denotes differences between sexually intact dogs of the CG and spayed dogs of the SG.

[4]SE of the difference (SED) for comparisons between diets.

[5]SED for comparisons between CG and SG.

[6]The interaction diet × group was not significant ($P > 0.10$) for any of the parameters.

[7]Estimated by subtracting 5.23 kJ/g digestible CP from the DE content.

procedure (PROC MIXED) including in the model the effects of diet, group, and the interaction (diet × group) as fixed effects and period and animal as random effects. Voluntary DMI (average of 5 d), ChFI (average of the 4 satiety tests), and activity level (average of the 5 d of ad libitum food intake recording) along with the basal concentration and the net and total AUC of blood hormones were analyzed including the fixed effect of phase and the interactions diet × phase, group × phase, and diet × group × phase in the model. According to the experimental design, the effect of sterilization is given by the interaction group × phase, denoting whether changes in the SG dogs from Ph.I to Ph.II differed from dogs in the CG. The time course of postprandial variation of blood hormones was analyzed including the sampling time after feeding (time = 15, 60, 120, 240, and 360 min) in the model as repeated measures subjected to animal by period by phase within group. Pairwise means comparisons were made using the Tukey's test. Correlations between ChFI and $nAUC_{0–240}$, $tAUC_{0–240}$, and the concentration of blood hormones at 240 min after feeding the experimental diets were calculated using the PROC CORR statement. Level of significance was set at $P < 0.05$, and $0.05 \leq P < 0.10$ was considered a trend. The results in the text are expressed as means ± SEM. In tables, the SE of the difference for comparisons between diets and between phases within the CG and the SG is provided.

RESULTS

All dogs remained healthy throughout the study. During Ph.I, the initial and final BW of SG dogs (still intact) was, in both measurements, 13.3 ± 0.6 kg and varied from 13.9 ± 0.5 to 13.8 ± 0.6 kg ($P > 0.10$) in CG dogs. During Ph.II, dogs' BW varied from 13.7 ± 0.6 to 13.9 ± 0.7 kg ($P > 0.10$) in spayed dogs of the SG and from 14.2 ± 0.6 to 14.1 ± 0.5 kg ($P > 0.10$) in intact dogs of the CG.

Dogs fed the HF diet showed a greater apparent digestibility of EE ($P < 0.001$), CP ($P < 0.01$), and GE ($P < 0.001$) than HC-fed dogs (Table 2). The DE and ME contents were also greater ($P < 0.001$) for the HF diet. Sterilization did not affect nutrient and energy digestibility, regardless of the composition of the diet (group and diet × group, $P > 0.10$).

Voluntary Food Intake, Challenge Food Intake, and Activity Level

Food intake and activity data are summarized in Table 3. Ad libitum distribution of the HF diet resulted in a greater voluntary DMI ($P < 0.05$) and ME intake ($P < 0.001$) compared with the HC diet. This diet effect was consistent between phases and was not affected by sterilization (diet × phase and diet × phase × group, $P > 0.10$). However, no differences between diets were found in postprandial ChFI ($P > 0.10$). Voluntary DMI tended to increase ($P = 0.056$) from Ph.I to Ph.II, with the increase being similar in CG and SG dogs (group × phase, $P > 0.10$). There was an increase ($P < 0.001$) in ChFI from Ph.I to Ph.II, and although the increase accounted for up to 33% in CG dogs and 56% in SG dogs, the interaction group × phase was not significant ($P > 0.10$).

Dogs' activity level during ad libitum feeding tended to be lower ($P = 0.057$) in dogs fed the HF diet

Table 3. Effect of diet and sterilization on voluntary DMI and ME intake, on the level of intake of a challenge food offered 4 h after feeding the experimental diets (on an as-fed basis), and on dogs' activity level (unit counts/min) during ad libitum feeding[1]

	Diet[2]		Sterilization[3]						*P*-value[6]		
			CG		SG						
Item	HC	HF	Ph.I	Ph.II	Ph.I	Ph.II	SED[4]	SED[5]	Diet	Phase	Group × phase
DMI, g/kg $BW0^{0.75}$	47.7	55.5	46.2	50.6	52.4	57.1	3.7	5.7	0.044	0.056	0.973
ME intake, kJ/kg $BW^{0.75}$	671	940	719	795	817	887	61	86	<0.001	0.057	0.960
ChFI,[7] g/kg $BW^{0.75}$	44.2	41.2	35.0	46.6	34.8	54.4	2.7	3.8	0.279	<0.001	0.147
Activity, counts/min	211	188	147	228	200	223	11	17	0.057	<0.001	0.022

[1]Values are least squares means for each diet ($n = 24$ and $n = 20$ for food intake and activity data, respectively) and for dogs of CG and the SG in Ph.I and Ph.II ($n = 12$ and $n = 10$ for food intake and activity level, respectively).

[2]HC = high-carbohydrate; HF = high-fat.

[3]CG = control group; SG = sterilization group; Ph.I = phase I; Ph.II = phase II. Sterilization effect was assessed as the difference from Ph.I to Ph.II in dogs of the CG that remained intact in both phases vs. dogs of the SG that were spayed in Ph.II (group × phase interaction).

[4]SE of the difference (SED) for comparisons between diets and between phases.

[5]SED for comparisons between phases within CG and SG.

[6]The interaction diet × group × phase was not significant ($P > 0.10$) for any of the parameters.

[7]ChFI = challenge food intake.

and increased ($P < 0.001$) from Ph.I to Ph.II, with the increase being lower in SG dogs than in CG dogs (group × phase, $P < 0.05$).

Basal Concentration of Blood Satiety Hormones

The significance of diet and sterilization effects on basal concentration of gut-derived hormones is shown in Table 4. Basal concentration of the studied hormones was not affected by diet ($P > 0.10$). The transition from Ph.I to Ph.II was associated with an increase ($P < 0.001$) of CCK and with a decrease ($P < 0.001$) of PYY levels in the fasted state, although these variations were independent of dog sterilization (group × phase, $P > 0.10$).

Postprandial Changes of Blood Satiety Hormones in Response to Diet

Active ghrelin concentration decreased ($P < 0.001$) from 15 to 60 min postprandially (Fig. 2A). The nadir was reached at 120 min with the HF diet (24.3% below the baseline) and at 360 min with the HC diet (18.9% below the basal value). Postprandial ghrelin suppression did not differ between diets (diet and diet × time, $P > 0.10$) and no differences were found in the $nAUC_{0–120}$ and $nAUC_{120–360}$ ($P > 0.10$; Table 5). Blood levels of CCK increased 15 min after feeding onward, with the response differing between dietary treatments (diet and diet × time, $P < 0.05$), with the HF diet leading to a lower and retarded maximal increase (118% of basal values at 240 min) with respect to the HC diet (208% of basal values at 120 min). The CCK increase was greater at 120 ($P < 0.01$) and 240 min ($P < 0.05$) in HC-fed dogs (Fig. 2B) and so were the $nAUC_{0–120}$ ($P < 0.01$) and the $nAUC_{120–360}$ ($P < 0.05$; Table 5). The increase in PYY after meal onset tended to be greater with the HC diet than with the HF diet from 120 min onward (diet × time, $P = 0.074$), and in HF-fed dogs, the maximal increase of PYY was delayed (53% of basal values at 240 min) in relation to HC-fed dogs (66% of basal values at 120 min). Dietary differences in postprandial PYY variation were significant at 120 min ($P < 0.05$; Fig. 2C), and the $nAUC_{120–360}$ tended to be greater ($P = 0.098$) with the HC diet (Table 5). Although insulin maximal increase was delayed in HF-fed dogs (97% of basal values at 360 min) in relation to HC-fed dogs (125% of basal values at 60 min), there was not an effect of diet on the postprandial evolution of insulin over 360 min (diet and diet × time, $P > 0.10$; Fig. 2D) or in the $nAUC_{0–120}$ and $nAUC_{120–360}$ ($P > 0.10$; Table 5). When the basal concentration was considered, dogs fed the HC diet had a greater ($P < 0.001$) $tAUC_{0–240}$ of CCK, whereas the $tAUC_{0–240}$ of active ghrelin, PYY, and insulin did not differ between diets ($P > 0.10$; Table 5).

Postprandial Changes of Blood Satiety Hormones in Response to Sterilization

Variations in blood concentration of all measured hormones from fasting to 360 min after feeding were not affected by sterilization (group × phase and group × phase × time, $P > 0.10$; Fig. 3), so changes in $nAUC_{0–120}$ and $nAUC_{120–360}$ of active ghrelin, CCK, PYY, and insulin from Ph.I to Ph.II did not differ between dog groups (group × phase, $P > 0.10$; Table 5). Sterilization did not affect the $tAUC_{0–240}$ of active

Table 4. Effect of diet and sterilization on basal concentration of blood ghrelin (active), cholecystokinin (CCK), total peptide YY (PYY), and insulin[1]

	Diet[2]		Sterilization[3]						*P*-value[6]		
			CG		SG						
Item	HC	HF	Ph.I	Ph.II	Ph.I	Ph.II	SED[4]	SED[5]	Diet	Phase	Group × phase
Ghrelin, pg/mL	597	550	561	615	533	585	37	52	0.215	0.163	0.974
CCK, p*M*	0.31	0.28	0.17	0.36	0.16	0.50	0.05	0.07	0.546	<0.001	0.146
PYY, pg/mL	608	584	804	525	625	431	61	85	0.691	<0.001	0.483
Insulin, microunits/mL	17.9	19.5	22.0	17.6	17.8	17.4	1.8	2.4	0.379	0.170	0.249

[1]Values are least squares means for each diet ($n = 24$) and for dogs of the CG and the SG in Ph.I and Ph.II ($n = 12$).

[2]HC = high-carbohydrate; HF = high-fat.

[3]CG = control group; SG = sterilization group; Ph.I = phase I; Ph.II = phase II. Sterilization effect was assessed as the difference from Ph.I to Ph.II in dogs of the CG that remained intact in both phases vs. dogs of the SG that were spayed in Ph.II (group × phase interaction).

[4]SE of the difference (SED) for comparisons between diets and between phases.

[5]SES for comparisons between phases within CG and SG.

[6]The interaction diet × group × phase was not significant ($P > 0.10$) for any of the parameters.

ghrelin, CCK, PYY, and insulin (group × phase, $P > 0.10$; Table 5).

Correlation between Challenge Food Intake and Blood Satiety Hormones

There was a negative correlation ($P < 0.05$) between ChFI and the $nAUC_{0–240}$ and $tAUC_{0–240}$ of insulin ($r = -0.353$ and $r = -0.364$, respectively) and a positive correlation between ChFI and the absolute concentration of active ghrelin at 240 min after feeding the experimental diets ($r = 0.357$, $P < 0.05$).

DISCUSSION

There is evidence that diets rich in protein and fiber improve satiety in dogs (Weber et al., 2007). In the present study, we aimed to evaluate the effect of feeding diets differing in the percentage of ME provided by fat or by nonstructural carbohydrates (namely as starch) but with the same percentage of ME provided as protein. Both diets were also formulated with a high level of fiber, similar to that conventionally used in diets designed for weight loss in dogs. The ME content was higher in the HF diet compared with the HC diet (16.9 vs. 14.1 MJ/kg DM, respectively) due to its higher fat content and its higher apparent digestibility of fat and protein compared with the HC diet. These ME values were close to those estimated from the chemical composition of the diets using the NRC (2006) approach (16.9 vs. 14.8 MJ/kg DM), which were actually used to establish the level of food offered. Therefore, energy restriction applied to dogs approximated the proposed 0.7 MER.

Effect of the Dietary Fat and Carbohydrate Content on Appetite Control

The greater DMI and ME intake of the HF diet when offered ad libitum is in line with Warwick (2003), who reported a linear dose response relationship between dietary fat and daily energy intake in rats. The greater DMI of the HF diet could be related to a higher motivation of dogs for diets containing a high fat:carbohydrate ratio. Preference for this macronutrient profile has been previously reported in dogs (Hewson-Hughes et al., 2013) and highlights a predominance of a hedonic-based mechanism of food intake control beyond an energy-based one (Erlanson-Albertsson, 2005). However, both diets induced a similar satiety response, as evidenced by the similar intake of the challenge food offered 4 h after the intake of the experimental diets at 0.7 MER. Previous studies have evidenced a major suppression of intake of a meal offered at 60 and 120 min after a low-fat compared with a high-fat isocaloric preload in rats (Gaysinskaya et al., 2007). Similarly, Blundell et al. (1993) showed in humans that breakfast supplementation with carbohydrates suppressed appetite 90 min later, whereas isocaloric supplementation with fat did not affect subsequent appetite. However, carbohydrate supplementation had no effect on the intake of a test meal given after 270 min (Blundell et al., 1993). Also, van der Klaauw et al. (2013) found no differences in the intake of a test meal offered 245 min after feeding a high-fat or a high-carbohydrate breakfast in humans. Therefore, it is possible that the major satiating capacity of carbohydrates may be predominately evidenced soon after food intake instead of several hours after feeding. Furthermore, Geoghegan et al. (1997) found even a major satiating capacity of fat 120 min after direct infusion of oleate and of a dextrose polymer into the small intestine. In addition, the 30% energy restriction applied to dogs in the current study during the challenge satiety

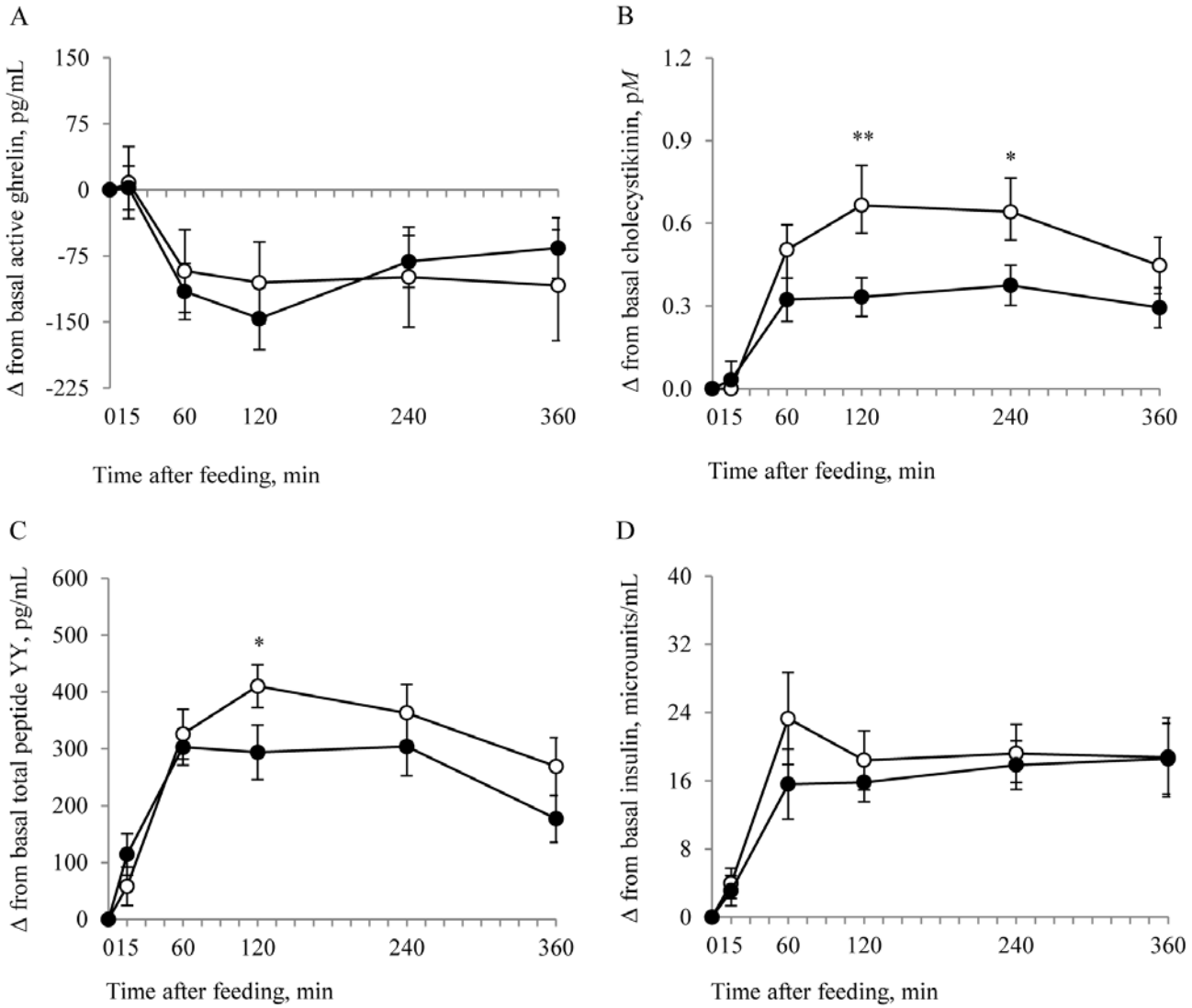

Figure 2. Postprandial variation of active ghrelin (A), cholecystokinin (B), total peptide YY (C), and insulin (D) from basal concentration in dogs fed the high-carbohydrate (-○-) or the high-fat (-●-) diet. Values are least squares means for $n = 24$ dogs per diet, with the SEM represented by vertical bars. Diet effect at each time point, $^{*}P < 0.05$, $^{**}P < 0.01$.

tests could have also affected the feeding response. Thus, in the study of Butterwick and Markwell (1997), dogs fed a low- or a high-fiber diet at 0.4 MER showed no differences in the intake of a common food offered 180 min later, whereas in the study of Bosch et al. (2009) dogs fed a high–fermentable fiber diet at 1 MER showed a trend toward a lower intake of a common food offered 360 min later in relation to dogs fed a low–fermentable fiber diet.

The activity values obtained in this study are similar to those reported by Hansen et al. (2007) using a similar device in healthy dogs. Interestingly, ad libitum feeding of the HF diet resulted in a reduction in dogs' activity level, which was probably the consequence of their higher level of intake (1.70-fold MER) in comparison with those offered the HC diet (1.22-fold MER).

Blood concentration of gut-derived hormones did not differ between diets in the fasted state and, as expected, food intake resulted in a suppression of active ghrelin and an increase of CCK, PYY, and insulin levels (Hameed et al., 2009). The similar ghrelin suppression found in HC- and HF-fed dogs is consistent with the study of Lubbs et al. (2010) in dogs, in which no differences between a load of fat and a load of maltodextrin were detected, and with 2 recent studies in humans comparing 2 isocaloric high-carbohydrate and high-fat meals (Gibbons et al., 2013; van der Klaauw et al., 2013), although it differs from previous ones reporting a higher suppression after high-carbohydrate diets (Monteleone et al., 2003; Yang et al., 2009). However, the lower postprandial increase of CCK and PYY found in dogs fed the HF diet contrasts with earlier studies reporting a higher elevation of CCK

Table 5. Effect of diet and sterilization on net and total area under the curve of blood ghrelin (active), cholecystokinin (CCK), total peptide YY (PYY), and insulin[1]

	Diet[3]		Sterilization[4]						*P*-value[7]		
			CG		SG						Group
Item[2]	HC	HF	Ph.I	Ph.II	Ph.I	Ph.II	SED[5]	SED[6]	Diet	Phase	× phase
$nAUC_{0–120}$											
Ghrelin, pg/mL	−71.4	−83.2	−95.0	−75.3	−51.0	−88.0	32.6	45.5	0.720	0.792	0.384
CCK, p*M*	0.41	0.22	0.38	0.46	0.18	0.23	0.07	0.09	0.009	0.307	0.829
PYY, pg/mL	266	229	223	232	279	256	37	51	0.314	0.284	0.669
Insulin, microunits/mL	16.1	11.2	15.3	14.2	15.0	10.1	3.9	5.5	0.227	0.453	0.625
$nAUC_{120–360}$											
Ghrelin, pg/mL	−105	−98.8	−112	−123	−62.8	−109	32.7	46.0	0.875	0.384	0.580
CCK, p*M*	0.59	0.34	0.73	0.57	0.27	0.29	0.09	0.13	0.010	0.417	0.332
PYY, pg/mL	364	253	279	320	235	400	66	92	0.098	0.129	0.353
Insulin, microunits/mL	19.1	17.1	20.2	16.9	21.3	13.9	3.4	4.7	0.562	0.122	0.549
$tAUC_{0–240}$											
Ghrelin, pg/mL	388	346	344	377	355	378	26	37	0.127	0.189	0.619
CCK, p*M*	0.83	0.58	0.76	0.92	0.38	0.75	0.07	0.10	<0.001	<0.001	0.112
PYY, pg/mL	937	845	1,059	886	870	748	54	76	0.103	0.013	0.641
Insulin, microunits/mL	35.5	32.5	38.0	35.3	34.5	28.1	3.2	4.4	0.363	0.150	0.553

[1]Values are least squares means for each diet (n = 24) and for dogs of the CG and the SG in Ph.I and Ph.II (n = 12).

[2]$nAUC_{0–120}$ = net area under the curve from 0 to 120 min; $nAUC_{120–360}$ = net area under the curve from 120 to 360 min; $tAUC_{0–240}$ = total area under the curve from 0 to 240 min.

[3]HC = high-carbohydrate; HF = high-fat.

[4]CG = control group; SG = sterilization group; Ph.I = phase I; Ph.II = phase II. Sterilization effect was assessed as the difference from Ph.I to Ph.II in dogs of the CG that remained intact in both phases vs. dogs of the SG that were spayed in Ph.II (group × phase interaction).

[5]SE of the difference (SED) for comparisons between diets and between phases.

[6]SED for comparisons between phases within CG and SG.

[7]The interaction diet × group × phase was not significant (P > 0.10) for any of the parameters.

in humans fed liquid diets containing corn oil as an individual nutrient with respect to glucose (Liddle et al., 1985) and of PYY in subjects fed a high-fat breakfast compared with subjects fed a high-carbohydrate breakfast (Gibbons et al., 2013). Greeley et al. (1989) also reported a higher PYY rise in dogs after an intraduodenal infusion of oleate compared with an intraduodenal infusion of AA, protein, or glucose. It is possible that the higher DM supply of the HC diet (to compensate its lower ME content) along with a comparatively faster gastric emptying rate in relation to dogs fed the HF diet (Mizuta et al., 1990) could have contributed to the higher CCK and PYY response of HC-fed dogs. In this respect, Juvonen et al. (2009) reported a major increase of blood CCK and PYY in subjects given a low-viscosity drink promoting a faster gastric emptying than a high-viscosity one. On the other hand, the presence of certain dietary fibers in the experimental diets, such as guar gum and cellulose, may have decreased lipase activity (Schneeman and Gallaher, 1980), which has been found to be key in the elevation of blood CCK (Feinle et al., 2003) and PYY (Degen et al., 2007). Furthermore, in the study of Degen et al. (2007), intraduodenal infusion of a CCK receptor antagonist also abolished PYY secretion in response to intraduodenal fat infusion.

Effect of Dog Sterilization on Appetite Control

There is strong evidence that dogs gain weight following sterilization (Robertson, 2003; McGreevy et al., 2005). Although this aspect was not the main focus of this work, we found scarce BW changes in spayed dogs and in control dogs during Ph. II (1.0 and −0.5% BW, respectively), likely because of the 30% energy restriction applied in different stages of the study. In line with the results obtained by Fettman et al. (1997) in cats, the apparent digestibility of nutrients was not affected by sterilization. Voluntary DMI, measured in the 5th (Period 1, Ph.II) and 11th week (Period 2, Ph.II) after sterilization, and ChFI, measured in the 8th (Period 1, Ph.II) and 14th week (Period 2, Ph.II), were not affected, in contrast to other studies in which energy intake increased after sterilization (Houpt et al., 1979; Jeusette et al., 2004). Discrepancies between experiments might be partly related to differences in the experimental design. For instance, in the study of Jeusette et al. (2004), the effect of sterilization was evaluated before and 6 mo after sterilization in the same group of dogs, although without us-

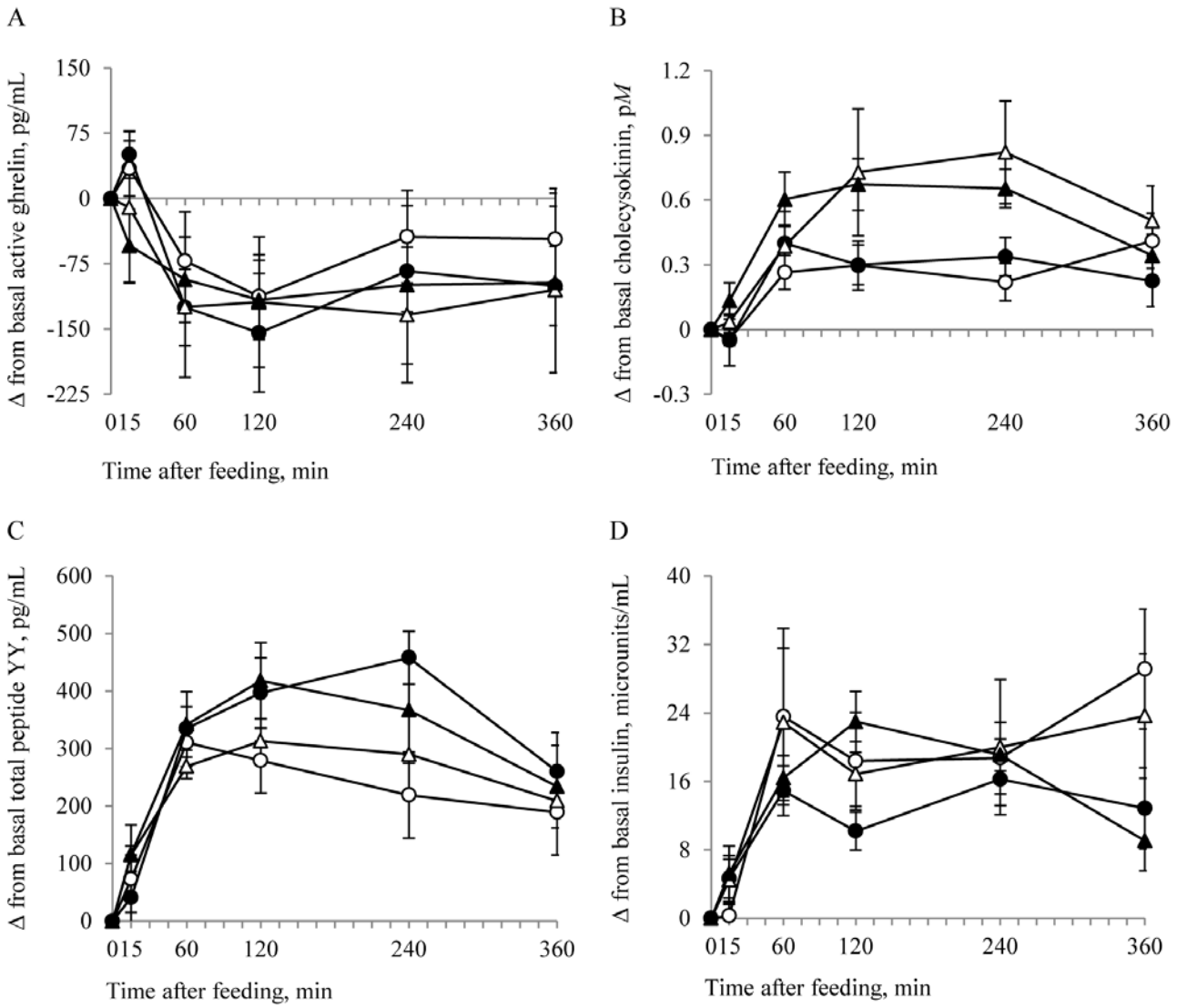

Figure 3. Postprandial variation of active ghrelin (A), cholecystokinin (B), total peptide YY (C), and insulin (D) from basal concentration in dogs of the sterilization group before sterilization (phase I; -○-) and after sterilization (phase II; -●-) and in dogs of the control group, which were intact in both phases (phase I; -Δ-) and (phase II; -▲-). Values are least squares means for $n = 12$ data per animal group in each phase, with the SEM represented by vertical bars. Sterilization effect (group × phase interaction) at each time point, $P > 0.10$.

ing a control group. Concerning the study of Houpt et al. (1979), in which a control group of sham operated dogs was included, spayed dogs showed a significant increase of food intake in the second week after sterilization, although not from the third to the fifth week or from the 13th to the 15th week after surgery. Interestingly enough, in this last study, the authors observed a lower daily energy intake in intact dogs during estrus in comparison with diestrus, being highest in anestrus, which indicates that the effect of the reproductive status on food intake control is chiefly related to the cyclic effect of estradiol rather than on a tonic or sustained one. Therefore, considering the reproductive physiology of the female dog in which the stage of estrus has a relatively short duration within the estrous cycle (Feldman and Nelson, 2004), it is arguable whether the lack of estradiol phasic effect in spayed dogs may cause a relevant impairment in food intake control, unlike cats or rats, which share a polyestrous sexual cycle. On this issue, according to a survey conducted by Heidenberger and Unshelm (1990) using a large database of keepers of spayed dogs, only 32% of these dogs showed changes in feeding behavior toward an increased food intake. There was a phase-related increase in ChFI and activity level in all dogs, irrespective of sterilization, most likely related to the decrease of external temperature from Ph.I to Ph.II (31 ± 2.6 and 15 ± 3.1°C, respectively), which corroborates the importance of including a control group in these kinds of studies. However, dogs that underwent sterilization did not significantly vary their activity level, unlike control dogs that increased their level of activity in Ph.II. In this regard, the survey of Heidenberger and Unshelm (1990) revealed a

decrease of activity in 18% of spayed dogs. Based on our findings, we cannot assert that the increased risk of BW gain occurring in dogs after sterilization is motivated by an increased level of energy intake but possibly by a decreased energy requirement, as reported by Jeusette et al. (2004), derived in part from a decreased physical activity.

Dog sterilization did not affect blood levels of gut-derived hormones in either the fasted or the postprandial state. As a matter of fact, most studies report an effect of estradiol on modulating the satiating and orexigenic effect of gut hormones rather than causing variations in their blood levels (Butera, 2010).

Within the studied hormones, only the postprandial levels of active ghrelin and insulin correlated with the feeding response 4 h after a meal. Indeed, insulin is a well-established satiety hormone (Schwartz et al., 1992), and a significant correlation between blood ghrelin and food intake previously has been found in humans (Gibbons et al., 2013). By contrast, blood levels of CCK and PYY were not correlated with the feeding response. At this respect, the satiating action of these 2 hormones at physiological levels is not consistently established (Reidelberger et al., 1989; Gibbons et al., 2013).

In conclusion, ad libitum feeding of a high-fat diet led to an increased daily energy intake, suggesting a predominance of a hedonic regulation of food intake over an energy-based one, and tended to reduce the activity level of dogs in comparison with a high-carboydrate diet. When dogs were offered a common diet 4 h postprandiallly, both diets induced a similar feeding response, reflecting a comparable short-term satiating capacity. Blood levels of CCK and PYY were influenced by the dietary fat and carbohydrate content, showing a higher increase in dogs fed a high-carbohydrate diet, but these changes were not related to changes in food intake 4 h after feeding. By contrast, blood levels of active ghrelin and insulin correlated with food intake but did not differ between diets. Dog sterilization was not associated with an impaired food intake control or with variations in blood gut-derived hormones from 5 to 15 wk after neutering but mainly with a decreased activity level in relation to sexually intact dogs.

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