JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Non-dioxin-like Polychlorinated Biphenyls (PCBs) and Chlordecone Release from Adipose Tissue to Blood in Response to Body Fat Mobilization in Ewe (*Ovis aries*)

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Supporting Information

ABSTRACT: Understanding how persistent organic pollutants (POPs) are released from adipose tissue (AT) to blood is a critical step in proposing rearing strategies hastening the removal of POPs from contaminated livestock. The current study aimed to determine in nonlactating ewes whether polychlorinated biphenyls (PCBs) and chlordecone are released from AT to blood along with lipids during body fat mobilization achieved through β-agonist challenges or undernutrition. β-Agonist challenges did not affect serum POP concentrations, whereas serum PCBs 138, 153, and 180 were readily increased in response to undernutrition. After 21 days of depuration in undernutrition, AT PCB 153 and 180 concentrations were increased concomitantly with a decrease in adipocyte volume, whereas AT chlordecone concentration was not different from that observed at the end of the well-fed contamination period. Thus, undernutrition may be of practical relevance for accelerating POP depuration unless it is combined with a strategy increasing their excretion pool.

KEYWORDS: *non-dioxin-like polychlorinated biphenyls, chlordecone, body fat mobilization, β-agonist, undernutrition, ewe*

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INTRODUCTION

Persistent organic pollutants (POPs), listed in the Stockholm convention,[1] have in common their deleterious effects on ecosystems and human health (i.e., carcinogenic, neurotoxic, reprotoxic, endocrine disrupting effects, ...),[2] their lipophilicity, and their low degradability properties, resulting in their high level of bioaccumulation within the environment to the food web.[3] Consumption of animal products such as fish, meat, milk, and eggs is one of the main routes of exposure to several POPs for humans.[4] To prevent this human health hazard, safety authorities have set regulatory limits concerning POP residue levels in food.[5] Nevertheless, several sanitary crises occurred due to accidental or environmental contaminations of animal products over these thresholds. These events caused large economic losses and induced profound social damage both on the farm and at the animal production chain levels. Because of the cumulative properties of POPs, the time needed for decontamination using classical herd management is too long, so that disposal rather than salvage of livestock is usually arranged.[6,7]

To face this worrying issue, there is a need to better understand how POPs are distributed and accumulated within livestock organisms and then eliminated, in order to develop innovative strategies aiming at hastening POP depuration from animal bodies. The first step consists of releasing lipophilic POPs from their storage site, the adipose tissue (AT), to the blood to make possible their metabolization or excretion. Some authors speculated that POPs would be released from AT to blood along with lipids, during body fat mobilization.[8] In ruminants, 2−4 weeks of undernutrition (i.e., dietary energy restriction) as a body fat mobilization strategy was successful in hastening dichlorodiphenyltrichloroethane (DDT) depuration from AT in dry ewes,[9] whereas this strategy failed to depurate dieldrin from AT in beef cattle.[10] However, in these two previous studies, body fatness was decreased, so that the question of a possible release of POPs from AT concomitant to short-term lipolysis was not addressed. Therefore, as far as we know, the respective impact of short-term AT lipolysis and of body fatness change on POP depuration cannot be established. Yet, the mechanisms involved in body depuration during fat mobilization should be understood for the design of relevant practical measures in the case of crises.

The aim of the current study was to test whether POPs are released from AT to blood along with lipids, over short- (1 h) or medium-term (3−28 days) body fat mobilization, achieved through β-adrenergic challenge or underfeeding, respectively, and to check whether this release occurs independent of body fatness decrease. The focus was on two types of POPs, with contrasting behaviors: non-dioxin-like polychlorinated biphenyls (NDL-PCBs), chlorinated chemicals formerly used as electrical insulation and flame retardant, which mainly accumulate in AT;[11] and chlordecone (CLD), an organochlorine pesticide, which mainly encounters hepatic sequestration.[12] In addition, these two categories of POPs are of great

Received: December 8, 2015
Revised: January 11, 2016
Accepted: January 13, 2016
Published: January 13, 2016

ACS Publications © 2016 American Chemical Society

DOI: 10.1021/acs.jafc.5b05817
J. Agric. Food Chem. 2016, 64, 1212−1220

concern because they were involved in several historical and current sanitary crises.[7,13,14]

MATERIALS AND METHODS

Chemicals. All of the organic solvents (Sigma-Aldrich, St. Louis, MO, USA) were of Picograde quality. Silica (Fluka, Sigma-Aldrich), Florisil (Promochem, LCG Standards, Molsheim, France), sodium sulfate (Merck, Darmstadt, Germany), and sulfuric acid (Sigma-Aldrich) were of superior analytical quality. Native and ^{13}C-labeled standards were purchased from Wellington Laboratories (Guelph, Canada) and Cambridge Isotope Laboratories (Tewksbury, MA, USA).

Contaminated Concentrate Manufacture. A contaminated feed was manufactured by incorporating contaminated oil into a concentrate mixture composed of dehydrated beet pulp, corn grain, and corn gluten meal [52:31:17, wt/wt dry matter (DM) basis]. Eight hundred and seventy-five grams of rapeseed oil was spiked with 4.38 mg of each of the six NDL-PCBs congeners (i.e., 26, 52, 101, 138, 153 and 180, purity ≥99%; Sigma-Aldrich) and with 438 mg of CLD (purity ≥ 99%; Sigma-Aldrich) before being mixed thoroughly by constant stirring (90 min, 20 °C) followed by sonication (20 min, 20 °C). Spiked oil was then homogeneously incorporated into 34 kg of concentrate mixture, using successively a knead mixer and a concrete mixer, resulting in expected concentrations of 129 μg of each NDL-PCB and 12.9 mg CLD/kg concentrate. Concentrate was presented as 9 mm pellets.

Animals and Diets. All experimental procedures were approved by an ethics committee (agreement CELMEA-2012-0015) and within the Agreement 00270.01 delivered by the French Ministry of Research and Higher Education. It was conducted in an adequate facility at Vandœuvre-lès-Nancy (Agreement B54-547-15, Lorraine, France). Four adult nonlactating and nonpregnant Romane ewes [*Ovis aries*; 4.5 ± 2.3 years old, 72.5 ± 6.4 kg body weight (BW), 3.4 ± 0.5 body condition score (BCS); mean ± standard deviation (SD)] were selected from the commercial farm of J. Bidon (Pulligny, France). The 63-day experiment was divided into three periods: two of oral exposure to POPs in positive (Expo+, days 1−35 of experiment) and then in negative dietary energy balance (Expo−, days 36−42), followed by a depuration period in negative energy balance (Depu−, days 43−63). During Expo+ and Expo−, diet was composed of 23% first-cut permanent grassland hay, 23% wheat straw, and 54% (DM basis) of pelleted concentrate, whereas during Depu− the diet comprised 37% hay, 37% straw, and 26% toasted sunflower meal. Restricted amounts of feed were individually distributed and adjusted weekly to cover 98% of maintenance energetic requirements (MER, 0.23 MJ of net energy/kg $BW^{0.75}$)[15] during Expo+, 35% during Expo−, and 42% during Depu−. During Expo+ and Expo− the proportions of contaminated and uncontaminated concentrates provided daily to each ewe were individually adjusted to achieve the target exposure level of 0.34 μg/kg BW/day of each NDL-PCBs and 34 μg/kg BW/day of CLD. All four ewes followed the above-mentioned sequence of dietary energy restriction, but only three ewes were exposed to contaminants, whereas the fourth ewe was kept as control without exposure to POPs. Diets were prepared and distributed in equal amounts twice daily at 9:00 a.m. and 4:00 p.m. Ewes were housed in individual boxes on wood shavings with individual feed bunks allowing controlled access to feed. They were allowed free access to water and received daily 20 g of minerals and vitamins premix. At the beginning of the study, they were shorn and orally given 15 mg/kg BW of anthelmintic (albendazole; Valbazen, Pfizer, New York, NY, USA).

β-Adrenergic Challenges. Intravenous β-adrenergic challenges were performed at days 35 (end of Expo+) and 42 (end of Expo−) of the experiment. At 6:00 p.m. the day before the adrenergic challenge, a catheter was fitted into the jugular vein of each ewe. At 8:00 a.m. the day after (i.e., 16 h after the last meal), isoproterenol (ISO, 4 nmol/kg BW of isoproterenol hydrochloride; Isuprel, Hospira, Brusse, Belgium) was intravenously injected over 20 ± 4 s (mean ± SD) through an extension (150 cm) attached to the catheter. Catheters were flushed with 25 mL of sterile saline to ensure complete infusion of ISO dose and to prevent contamination of subsequent blood samplings. Morning feeding was delayed until after the last blood sample was collected.

Sampling, Measurements, and Chemical Analyses. *Feed and Feces.* The amounts of feed distributed were recorded daily. No refusal was observed over the entire study. Subsamples of feeds were collected weekly and composited monthly for the determination of DM (103 °C, 24 h) and for the entire study for other analyses. After feedstuffs were ground and sieved through a 0.9 mm screen, they were analyzed according to standard methods for DM, ash, crude protein, neutral- and acid-detergent fibers, starch (only for concentrate), ether extract,[16] acid-insoluble ash,[17] in vitro pepsin−cellulase digestibility, and in vitro 1 h nitrogen degradability,[18] as well as NDL-PCBs and CLD concentrations (see below). The feeds' contents of energy and protein truly digestible in the small intestine were calculated using the INRA Prevalim software (version 3.23, Educagri éditions, Theix, France, 2006), and the ash, crude protein, acid-detergent fibers, ether extract contents, in vitro pepsin−cellulase digestibility, and 1 h nitrogen degradability.

Fecal outputs were estimated using acid-insoluble ash as marker,[17] analyzed in representative samples of feces individually and entirely collected over 48 h, at days 30−31 and 58−59 of experiment. Dried fecal samples (80 °C, 48 h) were ground and sieved through a 0.9 mm screen and analyzed for total and acid-insoluble ashes[17] and ether extract.[16]

Body Characterization. Individual BW was recorded weekly, BCS was assessed by palpation of the lumbar region by trained scorers, and subjective estimates were reported on a 0−5 scale[19] at days 1, 14, 28, 35, 42, 49, and 63 of experiment. Body fat mass was estimated by the deuterium oxide (D_2O) dilution space technique[20] at days 30 and 58 of the experiment. Briefly, after they were individually weighed, ewes were injected with 0.2 g of D_2O/kg BW (Euriso-top, Saint-Aubin, France) in the jugular vein through a catheter at 9:00 a.m. Six blood samples were collected in tubes containing lithium-heparin, immediately before deuterium oxide injection and 5, 7, 29, and 31 h after. Plasma was separated by centrifugation (1400*g*, 15 min, 4 °C) and subjected to deproteinization by filtration through centrifuge tubes of 10 kDa. Filtrates were then analyzed for $^2H/^1H$ enrichment by isotope ratio mass spectrometry. The D_2O dilution space, corresponding to the amount of D_2O injected divided by the concentration of D_2O at zero injection time (obtained by extrapolation of the exponential regression between plasma D_2O concentration and sampling time), was combined with BW at injection to determine body lipid mass using the equation proposed by Bocquier et al.[21] [body lipid mass (kg) = 0.863 BW − 0.865 D_2O space −9.1; R^2 = 0.92, residual SD = 1.40 kg].

Blood Sampling. Blood samples were collected from a jugular vein immediately prior to the initiation of β-adrenergic challenge at days 35 and 42 (−5 min) and at +5, +10, +15, +20, +30, and +60 min relative to ISO administration and at 8:00 a.m. before the morning feeding on days 35, 38, 42, 45, 49, 56, and 63 of the experiment. For each sampling time, subsamples were collected in tubes containing lithium-heparin and kept on ice, and plasma was separated within 1 h by centrifugation (1400*g*, 15 min, 4 °C) and frozen at −20 °C. Other subsamples were collected in tubes without preservative, allowed to clot (2 h, 20 °C), maintained at 4 °C during 24 h before serum was separated by centrifugation (1400*g*, 15 min, 4 °C), and frozen at −20 °C. Nonesterified fatty acids (NEFA), as well as glycerol and triglycerides (TG), were analyzed by spectrophotometry in plasma (Wako NEFA HR2 kit; Oxoid, Dardilly, France; and TR0100 kit; Sigma-Aldrich, respectively). Serum was analyzed spectrophotometrically for total lipids (HB018 kit; Cypress Diagnostics, Langdorp, Belgium) and for NDL-PCBs and CLD (see below).

Adipose Tissue. Pericaudal subcutaneous AT biopsies were performed on days 35, 42, and 63 of the experiment for adipocyte cellularity measurements and POP analyses. Skin was incised (5 cm) with a scalpel under local subcutaneous anesthesia (5 mL of 2% lidocaine; Lurocaïne, Vétoquinol, Lure, France). Adipose tissue was harvested at 5−8 cm above the tail head insertion, from the left and right sides alternately. Following the biopsy, incision was sutured and

DOI: 10.1021/acs.jafc.5b05817
J. Agric. Food Chem. 2016, 64, 1212−1220

Table 1. Intakes, Fecal Outputs, Body Measurements, and Plasma Metabolite Profiles of Ewes Exposed to Non-dioxin-like Polychlorinated Biphenyls (NDL-PCBs) and Chlordecone (CLD)[a]

item	period[b]			SEM	*P* value[c]
	Expo+	Expo−	Depu−		
intake[d] (g/day)					
total dry matter	967 a	343 c	606 b	42.5	<0.001
organic matter	905 a	317 c	559 b	40.1	<0.001
fat	26 a	12 b	11 b	1.2	<0.001
Σ 6 NDL-PCBs[e] (μg/day)	134.1 a	129.4 a	0.7 b	6.96	<0.001
CLD (μg/day)	2,075 a	2,012 a	0 b	108.4	<0.001
fecal output[f] (g/day)					
total dry matter	400	ND[g]	320	39.2	0.18
organic matter	321	ND	260	28.9	0.17
fat	10 a	ND	6 b	1.7	0.02
body measurement[h]					
body weight (BW, kg)	72.1 a	69.5 b	69.1 b	3.94	0.02
body condition score (0−5)	3.2 a	3.0 ab	2.9 b	0.25	<0.01
adipocyte diameter[i] (μm)	93	87	79	3.9	0.09
adipocyte volume[i] (pL)	407	354	258	118.8	0.14
body fat mass (kg)	15.7	ND	13.8	3.78	0.15
body fatness (% BW)	21.2	ND	19.8	4.06	0.54
balance[d] (% of maintenance requirements)					
energy	98 a	35 b	42 b	3.0	<0.001
PDI[j]	105 a	36 c	71 b	3.5	<0.001
plasma metabolite profile[k]					
nonesterified fatty acids (μM)	248 b	729 a	568 a	83.3	<0.01
glycerol (mg/dL)	0.31 b	0.52 ab	0.69 a	0.137	0.05
triglycerides (mg/dL)	13.9 a	4.4 b	3.6 b	1.63	<0.001
total lipids[l] (mg/dL)	252	264	283	11.9	0.09

[a]Means within a row with different letters (a−c) differ at $P \leq 0.05$. [b]Nonlactating and nonpregnant ewes ($n = 3$) received a sub-ad libitum diet composed of grass hay, wheat straw, and concentrate mixture, contaminated with NDL-PCBs [mean 0.31 (standard deviation 0.01) μg/kg body weight/day of each PCB 28, 52, 101, 138, 153, and 180] and CLD (28.9 μg/kg BW/day), and covering 98% of maintenance energy requirements (MER) over 35 days (exposure well-fed period, Expo+), followed by 7 days (from day 36 until day 42 of experiment) at 35% of MER (exposure underfed period, Expo−). Thereafter, ewes received a sub-ad libitum diet composed of grass hay, wheat straw, and toasted sunflower meal, without added NDL-PCBs or CLD, and covering 42% of MER over 21 days (from day 43 until day 63 of experiment; depuration underfed period, Depu−). [c]When included in the statistical model (i.e., intake, BW, body condition score, balance, and plasma metabolic profile), week within period effect was never significant ($P > 0.10$), except for plasma nonesterified fatty acids ($P = 0.08$). [d]Individual forage and concentrate intakes were recorded daily, and corresponding data where computed by week ($n = 9$ weeks). [e]Sum of PCBs 28, 52, 101, 138, 153, and 180. [f]Determined over 48 h at days 30−31 (Expo+) and 58−59 (Depu−) of the experiment. [g]Not determined. [h]Measured every week for BW ($n = 9$), at days 1, 14, 28, and 35 (Expo+), 42 (Expo−), and 49 and 63 (Depu−) of experiment for body condition score, at the end of each period (i.e., days 35, 42, and 63, for Expo+, Expo−, and Depu−, respectively) for adipocyte cellularity and at days 30 (Expo+) and 58 (Depu−) for body fat mass estimation by the deuterium oxide dilution space technic. [i]Adipocyte cellularity was determined from pericaudal subcutaneous adipose tissue. [j]Protein truly digestible in the small intestine.[14] [k]Measured at days 35 (Expo+), 38 and 42 (Expo−), and 45, 49, 56, and 63 (Depu−) of experiment. [l]Measured in serum.

treated with aluminum (Aluspray, Vétoquinol), and ewes received an intramuscular injection of antibiotics (0.1 mL/kg BW of 11% benzyl-penicillin; Pénijectyl, Virbac, Carros, France). A subsample of approximately 50 mg of AT was kept on physiological saline at 39 °C for <30 min and fixed by osmium oxide tetroxide (Euromedex, Souffelweyersheim, France) during 4 days. The fixed adipocytes were observed under a microscope, and average diameter and volume were determined using the software Visilog 6.7 (FEI Visualization Sciences, Merignac, France), as described by Bocquier et al.[21] Additionally, a subsample of 5−10 g of AT was frozen at −20 °C, lyophilized, and finely ground under liquid nitrogen pending POP analyses (see below).

Polychlorinated Biphenyl and Chlordecone Analyses. The analysis of NDL-PCBs in feedstuffs and AT was performed at the UR AFPA (Université de Lorraine, Vandœuvre-lès-Nancy, France), according to the method of Delannoy et al.[22] using gas chromatography coupled with mass spectrometry, whereas serum NDL-PCBs were analyzed at the LEAE-CART (Université de Liège, Liège, Belgium), as described by Debier et al.[23] using gas chromatography coupled with an electron-capture detector (GC-ECD). The determination of CLD concentration in feedstuffs and AT was performed at the Laboratoire Interdépartemental d'Analyses du Morbihan (LDA56, Lorient, France) for feedstuffs according to the method of Bordet et al.[24] using gas chromatography coupled with a triple-quadrupole mass spectrometer and for AT according to the POP09 ANSES method as described by Jondreville et al.[25] using liquid chromatography coupled with mass spectrometry in tandem. Serum CLD analysis was performed at the LEAE-CART according to the method of Multigner et al.[26] using GC-ECD. For NDL-PCBs, limits of quantifications were 0.2, 0.06, and 0.3 ng/g fresh weight for feedstuff, serum, and AT, respectively. For CLD they were 1, 0.06 and 2 ng/g fresh weight, respectively.

Calculations and Statistical Analyses. For β-adrenergic challenge data, maximal responses of blood metabolites and POP concentrations were calculated for each ewe by subtracting the baseline concentration (−5 min before ISO injection) to the maximal concentration observed after injection. The overall change during the 60 min sampling was estimated by calculating the area under the response curve and above the baseline (summation of the area between successive pairs of concentration−time coordinates).

Statistical analyses were performed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA; 2012). For β-adrenergic

DOI: 10.1021/acs.jafc.5b05817
J. Agric. Food Chem. 2016, 64, 1212−1220

challenge data, a model for repeated measures was used and included the period (Expo+ and Expo−) and the time relative to the ISO injection (repeated measure) as fixed effects, the ewe as a random effect, and a spatial power covariance structure. For the maximal response and the area under the curve, the model included only the period as fixed effect and the ewe as random effect. For data collected along the 63 days of the experiment, a model suitable for repeated measures was used, which included the period (Expo+, Expo−, and Depu−) and the day within period (repeated measures, except for fecal outputs, adipocyte cellularity, body fat mass, and POP concentrations in AT) as fixed effects and the ewe as random effect, using a spatial power covariance structure. Logarithmic transformation (base 10) of experimental data was performed when required (only for plasma NEFA) to comply with the assumptions of normality and homoscedasticity of residuals. In this case, least-squares means and standard errors of the mean were calculated from untransformed values, whereas declared P values reflect statistical analysis of transformed data. Period and time differences were declared significant at $P \leq 0.05$. Trends toward significance were considered at $0.05 < P \leq 0.10$. Values reported are least-squares means and standard error of the mean.

RESULTS

Characterization of the Diets, Intake, Fecal Output, and Energy Balance. The chemical and nutritional characteristics of feedstuffs are presented in Table S1. The concentrations of PCBs and CLD in the contaminated concentrate were slightly lower than expected (−10 ± 3% and −16%, respectively). PCBs were quantified in all other feedstuffs (hay, straw, sunflower meal) at levels on the order of 1000 times lower than in the contaminated concentrate, whereas CLD was not detected (Table S1).

By design, total DM and nutrient intakes were the highest ($P < 0.001$) and protein and energy balances were close to 0 during Expo+. Total DM intake was lower ($P < 0.001$) during Expo− than during Depu−, but due to the shift in diet composition, energy balance was equivalent ($P = 0.18$) and was negative in both cases (on average 39% of MER; Table 1 and Table S2). During Expo+ and Expo−, the daily intakes of PCBs were between 20.7 and 23.8 μg/day and intake of CLD was 2043 μg/day (Table S2). Therefore, over the 42 days of exposure, ewes received 13.0 ± 0.6 μg/kg BW of each NDL-PCB congener and 1207 μg/kg BW of CLD, which was slightly lower than the targeted 14.4 μg/kg BW for each PCB and 1440 μg/kg BW for CLD. During Depu−, the daily intake was 0.13 ± 0.05 μg/day for each PCB and 0 μg/day for CLD (Table S2).

Compared to Expo+, fat fecal output was lower ($P = 0.02$) during Depu−. In contrast, total DM and organic matter fecal outputs remained unaffected ($P > 0.10$; Table 1) due to decreased apparent digestibility of DM from 59% during Expo+ to 47% during Depu− and from 65 to 53% for organic matter.

Effects of β-Adrenergic Challenges. Isoproterenol injection induced fast and transient increases ($P < 0.05$) in both plasma NEFA and glycerol concentrations. These responses were numerically higher during the underfed than during the well-fed period (Table S3 and Figure 1). Conversely, serum NDL-PCBs and CLD concentrations were not affected ($P > 0.10$) by ISO injection (reported only for PCB 153 and CLD in Figure 1).

Effects of Undernutrition. *Blood Metabolites and Body Fatness.* Short-term energetic undernutrition (i.e., 3 and 7 days, Expo+ vs Expo−) induced a sharp increase ($P \leq 0.05$) in plasma NEFA, which remained high thereafter over the 21 days of the Depu− undernutrition period. Glycerol concentration

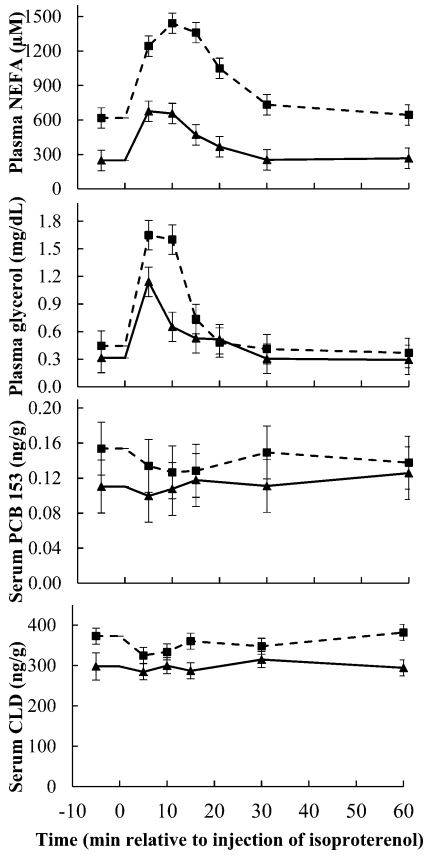

Figure 1. Time-dependent responses to β-adrenergic challenges (injections of isoproterenol, 4 nmol/kg of body weight) of blood plasma nonesterified fatty acids (NEFA) and glycerol and of serum polychlorinated biphenyl (PCB) 153 and chlordecone (CLD) concentrations in nonlactating and nonpregnant ewes ($n = 3$) exposed to non-dioxin-like PCBs and CLD, when well-fed [98% of maintenance energy requirements (MER), day 35; ——▲——], or underfed (35% of MER, day 42; - -■- -). Each point represents the least-squares mean, and error bars indicate the standard error of the mean. Effects of time, period, and time × period were significant ($P < 0.05$) for NEFA and glycerol, and only the period effect was significant ($P < 0.05$) for PCB 153 and CLD.

was also increased, but only significantly ($P = 0.05$) after 9 days of undernutrition. Conversely, plasma TG concentration dropped widely ($P < 0.001$) after only 3 days of undernutrition and was even lower thereafter (Figure 2). After 7 days (Expo+ vs Expo−), body fat mobilization did not influence ($P > 0.10$) body fatness, assessed through BCS or adipocyte diameter and volume. Conversely, after 28 days of undernutrition, both BW and BCS were decreased ($P \leq 0.02$), as well as adipocyte diameter and volume and body fat mass (estimated based on D_2O dilution space), which were numerically reduced by 15, 37, and 12%, respectively ($P \leq 0.15$; Table 1).

Polychlorinated Biphenyls and Chlordecone Kinetics in Serum and Adipose Tissue. All PCBs were quantified in all samples of serum, whereas PCBs 28 and 52 were not quantifiable in AT due to a too high noise-to-signal ratio in this part of the chromatogram. Before oral exposure to POPs (day 0 of experiment), serum PCB concentrations were low (Table 2). These concentrations remained constant for the uncontaminated ewe during the experiment (results not shown). In accordance, the AT concentrations of PCBs in this control ewe were also low at days 35 and 63 of experiment:

DOI: 10.1021/acs.jafc.5b05817
J. Agric. Food Chem. 2016, 64, 1212−1220

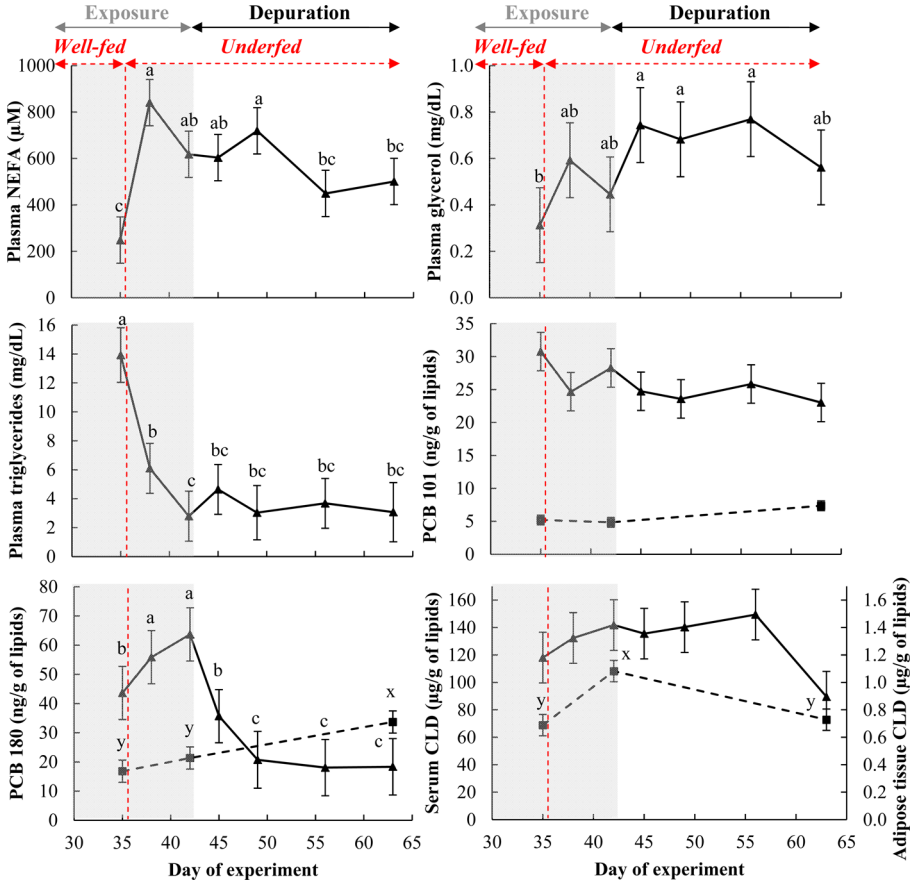

Figure 2. Time pattern kinetics of nonesterified fatty acids (NEFA), glycerol, and triglyceride concentrations in plasma and of polychlorinated biphenyls (PCBs) 101 and 180 and chlordecone (CLD) concentrations in serum (——▲——) and in pericaudal subcutaneous adipose tissue (- -■- -) of nonlactating and nonpregnant ewes exposed to non-dioxin-like PCBs and CLD over 42 days (gray background) and therefore depurated for 21 days (from day 43 until day 63 of experiment; white background) and either well-fed [98% of maintenance energy requirements (MER), from day 0 until day 35] or underfed (35−42% of MER, from day 36 until day 63). Each point represents the least-squares mean, and error bars indicate the standard error of the mean. *P* value for period and day within period effects are reported in Tables 1 and 2. Means with different letters (a−c) differ at $P \leq 0.05$ for plasma metabolites profile and serum PCBs and CLD concentrations. Means with different letters (x, y) differ at $P \leq 0.05$ for adipose tissue PCBs and CLD concentrations.

on average 3.4 ± 0.7, 2.6 ± 1.1, 7.9 ± 2.4, and 3.3 ± 0.4 ng/g of lipids (mean ± SD) for PCBs 101, 138, 153, and 180, respectively. Chlordecone was quantified in all samples of serum and AT except for the control ewe and for the initial sampling for the three exposed ewes.

For the three contaminated ewes, at the end of Expo+, serum concentrations of PCBs 138, 153, and 180 were increased by 4−10-fold ($P < 0.001$) compared to the initial levels. Conversely, serum PCB 52 and 101 concentrations were not affected ($P \geq 0.10$) by contamination. Serum PCB 138, 153, and 180 concentrations were increased ($P \leq 0.02$) within 7 days of undernutrition (Expo+ vs Expo−), an effect that already tended to be significant ($P \leq 0.07$) after only 3 days (Table 2; Figure 2; Figure S1). Initial decreases in serum concentrations of PCBs 138, 153, and 180 were observed between the end of the Expo− period and the seventh day of the Depu− period (−60% on average, $P < 0.01$). After this initial drop, serum PCB concentrations remained unchanged ($P > 0.10$) until the end of the 21-day Depu− period. Conversely, PCB 153 and 180 concentrations in AT were higher at the end of Depu− compared to the ends of the Expo+ and Expo− periods ($P \leq 0.03$; Table 2; Figure 2; and Figure S1).

At the end of Expo+, serum and AT CLD concentrations were 118 and 0.69 μg/g of lipids, respectively. Within 7 days of undernutrition (Expo+ vs Expo−), AT CLD concentration was increased (+57%, $P = 0.03$), whereas only a numerical increase was observed for serum CLD concentration (+20%, $P = 0.23$). During the Depu− period, serum CLD concentration remained unchanged ($P > 0.15$) over the first 14 days, before being decreased ($P < 0.01$) from the 14th to the 21st day, whereas AT CLD concentration was decreased ($P = 0.03$) from the beginning to the end of the depuration period (Table 2; Figure 2).

■ DISCUSSION

A better understanding of how POPs are released from storage tissues to blood is a critical step in proposing breeding strategies aiming at hastening the removal of POPs from contaminated animals. Novel aspects of the current investigation include an assessment of the effects of lipomobilization induced by β-adrenergic challenge and undernutrition on the lipophilic POP release from AT to blood.

Effects of β-Adrenergic Challenges. Transient increase in AT lipolysis due to ISO injection was expected, as ISO is a nonselective β-agonist stimulating the β-adrenoceptors and therefore enhancing the hormone-sensitive lipase, which catalyzes the breakdown of TG from AT into NEFA and glycerol.[27] Moreover, the observed higher lipolytic response

DOI: 10.1021/acs.jafc.5b05817
J. Agric. Food Chem. 2016, 64, 1212−1220

Table 2. Non-dioxin-like Polychlorinated Biphenyls (NDL-PCBs) and Chlordecone (CLD) Concentrations in Serum and Pericaudal Subcutaneous Adipose Tissue of Ewes[a]

item	initial value[b] (mean ± SD)	period[c]			SEM	*P* value	
		Expo+	Expo−	Depu−		period	week (period)
serum (/g of lipids)[d]							
PCB 28 (ng)	54.3 ± 19.9	200.9 a	88.5 b	101.0 b	30.22	0.03	0.19
PCB 52 (ng)	156 ± 57	145.2	86.7	131.7	27.82	0.40	0.23
PCB 101 (ng)	32.8 ± 4.7	30.8	26.5	24.3	2.22	0.15	0.41
PCB 138 (ng)	0.9 ± 1.6	2.5 b	7.3 a	1.0 b	1.07	0.02	0.61
PCB 153 (ng)	9.3 ± 7.6	44.3 a	52.2 a	30.1 b	7.74	<0.001	0.02
PCB 180 (ng)	1.0 ± 1.0	43.6 b	59.8 a	23.2 c	8.78	<0.001	0.09
Σ 6 NDL-PCBs (ng)	254 ± 29	466 a	319 b	307 b	31.2	0.01	0.19
CLD (μg)	not detected	118	137	129	16.3	0.43	0.10
adipose tissue (/g of lipids)[e]							
PCB 101 (ng)	ND[f]	5.2	4.8	7.4	0.74	0.19	
PCB 138 (ng)	ND	10.4	11.4	16.5	2.51	0.65	
PCB 153 (ng)	ND	24.2 b	28.8 b	58.8 a	5.68	<0.001	
PCB 180 (ng)	ND	16.8 b	21.3 b	33.7 a	3.80	0.03	
Σ 4 NDL-PCBs[g] (ng)	ND	56.0 b	65.8 b	117.0 a	12.28	0.01	
CLD (μg)	ND	0.69 b	1.08 a	0.73 b	0.078	0.03	

[a]Means for period within a row with different letters (a−c) differ at $P \leq 0.05$. [b]Day 0 of experiment before NDL-PCBs and CLD exposure, not included in statistical analyses. [c]See footnote *b* of Table 1. [d]Measured at days 35 (Expo+), 38 and 42 (Expo−), 45, 49, 56, and 63 (Depu−) of experiment. [e]Measured at the end of each period (i.e., days 35, 42, and 63, for Expo+, Expo−, and Depu−, respectively). PCBs 28 and 52 were not quantified in adipose tissue because of a too high signal-to-noise ratio in this area of the chromatograms. [f]Not determined. [g]Sum of PCBs 101, 138, 153, and 180.

due to ISO injection in underfed than in well-fed ewes is in accordance with previous results obtained in lactating ewes[28] or in dry cows[29] and could be explained by the fact that AT lipolytic potential is primarily regulated according to physiological needs, which are higher in animals in negative energy balance.[27]

Serum NDL-PCBs and CLD concentrations were not affected by ISO injection. Similarly, incubating bovine AT cultured in vitro with epinephrine (a β-agonist) did not induce any release of β-carotene,[30] another lipophilic molecule stored in AT along with TG.[31] Thus, conversely to our initial hypothesis, our results suggest that NDL-PCBs are not released from AT to blood in the case of β-adrenergic challenge, even when the lipolytic response is maximized due to negative energy balance. Alternatively, NDL-PCBs might be quickly re-incorporated in AT after their release so that no increase in serum concentrations could be observed. This last assumption is supported by the fact that POPs administrated intravenously are known to be cleared from blood very rapidly. In fact, radiolabeled hexachlorobenzene (another lipophilic POP) contained in chylomicrons intravenously injected to rat is cleared from the blood in <10 min and 2-fold more quickly than TG.[32]

Effects of Undernutrition. In response to energetic dietary shortage, ewes mobilized quickly their body fat stores as ascertained by sharp increases in plasma NEFA and glycerol and decrease in plasma TG, which ultimately induced a decrease in body fatness after 28 days. Those adaptive responses of ewes to undernutrition are in full agreement with the literature.[27,33] Thus, in dry Lacaune ewes, increases in plasma NEFA concentration were also observed after only 2−4 days of undernutrition,[27,33] whereas TG dropped sharply subsequent to 7 days of undernutrition down to 22% of MER in Lacaune ewes.[34] Moreover, 166 days of undernutrition at 40% of MER in Romane ewes decreased BW at a weekly rate of −1 kg, BCS at −0.05 point, and body fat mass at −0.36 kg,[35] compared to −1.3 kg BW, −0.08 BCS, and −0.48 kg of body fat mass per week in the current study. When lactating Bergamasca ewes were in negative energy balance over the 42 first days of lactation, adipocyte diameter of the subcutaneous AT from the rump was reduced by 30 μm[36] compared to 14 μm in 28 days in the present study.

The observed +300%, +36%, and +46% increases in serum PCBs 138, 153, and 180, respectively, subsequent to 7 days of undernutrition (Expo+ vs Expo−) were disconnected from body fatness and AT NDL-PCB concentrations changes. These observations are in accordance with a previous study on chickens, where 2−4 days of complete food deprivation increased blood DDT content by +340% compared to a full-fed treatment.[37] Such increases in serum PCB concentrations may originate, at least partly, from two distinct mechanisms related to short-term regulations of lipid dynamics:

(i) An increased release of NDL-PCBs from AT to blood due to lipolysis. Accordingly, Patterson[38] reported in sheep that the plasma concentration of xanthophylls (other lipophilic molecules stored in AT) was sharply increased by complete food deprivation over 2−4 days. As plasma xanthophylls and NEFA increases were closely related, the author suspected a direct relationship between body fat mobilization and xanthophyll release from AT to blood.[38]

(ii) A decrease of NDL-PCB uptake by AT from lymph and blood, due to a decrease in lipogenesis and more specifically in lipoprotein lipase activity. Lipoprotein lipase of AT is responsible for the hydrolysis of circulating TG associated with chylomicrons and very low density lipoproteins, which are the main vehicle of PCBs in lymph and blood.[39] As lipoprotein lipase activity is known to be decreased within only 2−4 days in response to undernutrition in ewe,[33] this may have a depressing effect on NDL-PCB uptake by AT. Accordingly, Kohan et al.[40] showed that incubating

DOI: 10.1021/acs.jafc.5b05817
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chylomicrons with lipase increased by 3-fold the DDT uptake by cultured rat adipocytes.

The observed 7-day initial drop, followed by a slower decrease in serum PCB 138, 153, and 180 concentrations after the cessation of oral exposure (Depu− period), is in accordance with a previous study on dairy goats.[41] It may originate from the buffering effect of rumen, which may cause delayed absorption of dietary constituents, including PCBs, by up to 7 days in ruminants.

More surprisingly, AT PCB 153 and 180 concentrations were higher at the end of the Depu− compared to the Expo+ and Expo− periods, despite the cessation of oral exposure. This puzzling result may be explained by the inability of animals to metabolize these NDL-PCBs and consequently to eliminate them, leading then to a close inverse relationship between the size of their storage compartment (i.e., AT) and their concentrations in it. Indeed, in nonlactating mammals, the almost exclusive route of depuration of highly lipophilic and poorly metabolized compounds such as PCBs 153 and 180 is the fecal lipid output.[42] However, in the present study the estimated amount of fecal lipids excreted was 126 g over the 21 days of Depu−, which represented only 0.8% of the estimated total body fat mass. Thus, the elimination of PCBs through this route was probably negligible. In the same period of time (21 days of Depu−), the adipocyte volume of pericaudal subcutaneous AT was decreased by 1.4-fold, which was remarkably close to the 1.6-fold average increase in PCB 153 and 180 concentrations in this AT. This direct inverse relationship between the size of the storage pool and the increase in its concentrations of PCBs was previously observed in contaminated chickens following a 21-day undernutrition depuration period at 50% of ad libitum intake.[43] In this latter study, compared to ad libitum diet, the 2.7-fold increase in whole carcass PCB concentration on fat basis was concomitant with the 2.5-fold decrease in carcass fat mass.[43]

The absence of observable effect of PCB oral exposure and of undernutrition treatment on the less chlorinated PCB 28, 52, and 101 concentrations in serum and AT is probably due to their higher degree of metabolization, especially in the liver, and consequently to their low bioaccumulation in animal tissues, as previously reported in dairy goats.[41,44]

The response of CLD to undernutrition was markedly different from the one of PCBs. Chlordecone concentration was increased by 1.6-fold in AT after 7 days of undernutrition (Expo+ vs Expo−) but was reduced during the depuration period, despite the decreased size of the AT compartment. As CLD is mainly sequestrated in the liver,[12] such modulations of AT CLD concentrations may be related to regulations in the physiology of splanchnic tissues rather than to the size of AT. Indeed, decreased liver size in response to short-term nutrient shortage has been previously described in sheep after 7 days of undernutrition.[45] A concomitant release of CLD stored in the liver and its subsequent redistribution toward other compartments including AT, but also serum (serum CLD concentration was increased by 1.2-fold), may therefore have occurred. However, this mechanism cannot be ascertained unless specific measurements are performed on the liver.

The observed unusual time pattern in CLD serum concentration during the depuration period, characterized by an initial 15-day plateau, was previously observed in growing pig.[46] Moreover, the decreases observed in serum (−40%) and AT (−32%) concentrations over the 21-day Depu− period were far lower than the −80% for serum and −87% for sternal subcutaneous AT reported in well-fed growing goat kids depurated for 21 days.[47] Such discrepancy could be explained either by a lower ability of ewes to metabolize CLD compared to goat kids or by a decrease in hepatic metabolization of CLD due to undernutrition in the present study. Accordingly, nutrient restriction is known to sharply decrease portal and hepatic blood flows, as well as liver weight.[48]

To conclude, <1 h of AT lipolysis induced by β-adrenergic challenge does not influence serum PCB and CLD concentrations, whatever the feeding level. Conversely, and in accordance with our initial hypothesis, body fat mobilization induced by undernutrition seems to elicit a fast (<1 week) release of poorly metabolized NDL-PCBs (i.e., 138, 153, and 180) from AT to blood. However, subcutaneous AT PCB 153 and 180 concentrations increased over the 21-day depuration period in undernutrition, as a consequence of their recalcitrance to metabolization and, in turn, to elimination, combined with the decrease in the size of AT (i.e., adipocyte volume) in response to medium-term body fat mobilization. To be efficient in depurating animals, undernutrition should be combined with a strategy increasing the fecal lipid output and consequently the POP excretion pool, such as the supplementation of the diet with nonabsorbable lipids. This combined strategy was tested with success for hastening the removal of PCBs in chickens.[43] Further studies are needed to assess its efficiency in larger animals such as ruminants, where only nonabsorbable lipid supplementation in well-fed growing lambs[49] or lactating cows and goats[50] was tested. With regard to CLD, which accumulates in the liver rather than in AT, undernutrition seems not to represent a valuable strategy because of its probable deleterious effect on liver size and metabolic activity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.5b05817.

Feedstuff chemical composition and POP concentrations (Table S1), ingredients and POP intakes (Table S2), response to β-adrenergic challenge of plasma NEFA and glycerol (Table S3), and time pattern kinetics of PCBs 138 and 153 (Figure S1) (PDF)

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Funding

This work was supported in part by the Conseil Régional de Lorraine under the call of research proposal Université/EPST − Région 2014 (contract Relations entre flux de polluants organiques persistants et flux de lipides à l'échelle de l'organisme de l'animal d'élevage − FLipPOP).

Notes

The authors declare no competing financial interest.

DOI: 10.1021/acs.jafc.5b05817
J. Agric. Food Chem. 2016, 64, 1212−1220

■ ACKNOWLEDGMENTS

We thank A. Fournier and M. Delannoy (UR AFPA, Université de Lorraine) for helpful discussion during the preparation of the contaminated oil, N. Besné and E. Ledru (INRA UE1295 Pôle d'Expérimentation Avicole de Tours, Nouzilly, France) for concentrate manufacture, P. Hartmeyer (UR AFPA, Université de Lorraine) for feeding and management of ewes, H. Toussaint and G. Rychen (UR AFPA, Université de Lorraine) for technical support during the β-adrenergic challenge procedures, L. Saffroy (clinique vétérinaire du Grémillion, Essey-lès-Nancy, France) for biopsies procedures, C. Grandclaudon, S. Poinsignon (UR AFPA, Université de Lorraine), and C. Adam (Université de Liège) for PCBs and CLD analyses, I. Constant and A. De La Torre Capitan (INRA, UMR1213 Herbivores, Saint-Genès-Champanelle, France) for adipocyte cellularity measurements, C. Brechet (INRA, UMR1137 Ecologie et Ecophysiologie Forestières, Champenoux, France) for plasma deuterium oxide analyses, J. A. A. Pires and A. Ferlay (INRA, UMR1213) for helpful discussions relative to β-adrenergic challenge procedures, and C. Jondreville (UR AFPA, INRA USC340) for helpful discussion during the preparation of the manuscript.

■ ABBREVATIONS USED

AT, adipose tissue; BCS, body condition score; BW, body weight; CLD, chlordecone; D_2O, deuterium oxide; DDT, dichlorodiphenyltrichloroethane; DM, dry matter; GC-ECD, gas chromatography coupled with electron-capture detector; ISO, isoproterenol; MER, maintenance energy requirements; NDL-PCBs, non-dioxin-like polychlorinated biphenyls; NEFA, nonesterified fatty acids; PCBs, polychlorinated biphenyls; POPs, persistent organic pollutants; SD, standard deviation; TG, triglycerides

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