

Macronutrient composition determines accumulation of persistent organic pollutants from dietary exposure in adipose tissue of mice

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Abstract

Accumulation of persistent organic pollutants (POPs) has been linked to adipose tissue expansion. As different nutrients modulate adipose tissue development, we investigated the influence of dietary composition on POP accumulation, obesity development and related disorders. Lifespan was determined in mice fed fish-oil-based high fat diets during a long-term feeding trial and accumulation of POPs was measured after 3, 6 and 18 months of feeding. Further, we performed dose–response experiments using four abundant POPs found in marine sources, PCB-153, PCB-138, PCB-118 and pp'-DDE as single congeners or as mixtures in combination with different diets: one low fat diet and two high fat diets with different protein:sucrose ratios. We measured accumulation of POPs in adipose tissue and liver and determined obesity development, glucose tolerance, insulin sensitivity and hepatic expression of genes involved in metabolism of xenobiotics. Compared with mice fed diets with a low protein:sucrose ratio, mice fed diets with a high protein:sucrose ratio had significantly lower total burden of POPs in adipose tissue, were protected from obesity development and exhibited enhanced hepatic expression of genes involved in metabolism and elimination of xenobiotics. Exposure to POPs, either as single compounds or mixtures, had no effect on obesity development, glucose tolerance or insulin sensitivity. In conclusion, this study demonstrates that the dietary composition of macronutrients profoundly modulates POP accumulation in adipose tissues adding an additional parameter to be included in future studies. Our results indicate that alterations in macronutrient composition might be an additional route for reducing total body burden of POPs.

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1. Introduction

Lipid-soluble persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethanes (DDTs), bioaccumulate through the food web and are stored in human adipose tissue. Although their use was banned in most countries several decades ago, DDTs and PCBs are still found at considerable levels in human adipose tissue due to their earlier widespread use and persistency [1–3]. Studies have reported an association between obesity and plasma levels of certain PCBs and pesticides [4–7],

suggesting a possible relationship between POP exposure and the current obesity epidemic as well as type 2 diabetes [7–10]. However, a causal relationship between POP exposure and obesity development has not yet been demonstrated, and conflicting data have been reported [11–14].

Repeated injections of PCB-153 [15] and PCB-77 [16] are reported to exacerbate obesity in mice. We have earlier observed that POPs of marine origin accumulate in adipose tissue concomitant with obesity development in rats [17] and mice [18]. These studies indicated a relationship between dietary POP exposure and development of obesity. However, as the different dietary POP levels were accompanied with altered macronutrient compositions in these experiments, it is impossible to discriminate between effects of POP exposure *per se* and effects associated with altered macronutrient composition. Moreover, as recent mouse studies have failed to demonstrate a direct correlation between POP dosage and obesity development [19,20], it is not obvious whether the observed differences in obesity development in these studies are nutrient dependent and/or POP load dependent.

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The ability of n-3 polyunsaturated fatty acids (PUFAs) to attenuate the action of PCBs in different cell systems is well documented [21]. Conversely, it has been suggested that the antiobesogenic effect of n-3 PUFAs is attenuated by POPs [17]. Such interactions may be of importance as fatty fish and fish oil are sources of both marine n-3 PUFAs and POPs, which generally increase concomitantly with the lipid level in seafood. Dietary constituents, such as linoleic acid [22], sucrose [23] and other high glycemic index carbohydrates [24], also interact with n-3 PUFAs and attenuate their antiobesogenic effect. Thus, different nutrients may interact with POPs, as well as with each other and thereby modulate whole body metabolism in a complex manner.

Accumulation of the highly chlorinated and nearly nonmetabolizable PCB-153 in adipose tissue of rats depends on whether adipose tissue is in steady state or whether it is expanding or shrinking [25–27]. To investigate the potential influence of dietary composition on POP accumulation and the potential link between accumulation of POPs in adipose tissue and obesity development, a series of animal feeding trials were performed. In several human studies, age exhibits the strongest correlation with tissue accumulation of POPs [11–13]. Therefore, we measured accumulation of POPs in adipose tissue in mice fed fish-oil-enriched diets with different macronutrient compositions for 6 and 18 months and determined the effect on metabolic parameters and lifespan. We further selected four of the most abundant POPs in adipose tissue (PCB-118, PCB-138, PCB-153 and pp'-DDE) for dose-response studies. We measured their deposition in adipose tissue and examined the correlation between accumulation in adipose tissue and obesity development in mice. Finally, we administered the selected four POPs through diets with different fatty acid composition and different protein:sucrose ratios to investigate possible interactions between POPs and feed composition.

2. Materials and methods

2.1. Animals

All animal handling and experimental protocols were approved by the Animal Experiment Inspectorate in Denmark and the Norwegian Animal Research Authority (FOTS ID nos. 4493, 3741, 3526, 3274, 3199 and 5358) and were conducted in accordance with the guidelines of the national authorities in compliance with the European convention for the protection of vertebrate animals used for experiments and other scientific purposes (Council of Europe, no. 123, Strasbourg, France, 1985). In all experiments, female C57BL/6J BomTac mice 3 weeks of age (Taconic Europe) were housed in groups of four mice per cage at room temperature (22°C) with a 12-h light/dark cycle. The animals had free access to water and were fed their respective diets (Table S1) *ad libitum*. Three independent experiments were performed. The animals were weighed and divided into the experimental groups ensuring an equal distribution and equal average body weight in all experimental groups before start.

In experiment 1, 150 mice were fed a low fat (LF) diet or a high fat diet based on fish oil with either a high content of sucrose (FOS) or protein (FOP) (Table S1). After 6 and 18 months of feeding, 10 mice from each group were euthanized and organs were collected (see 2.5. Tissue sampling). The remaining mice ($n=30$) from each group were kept on the diet until 50% of the mice had died, which happened after 110, 91 and 97 weeks of feeding in the LF, FOS and FOP groups, respectively. At this time point, remaining mice were euthanized and organs were collected (see 2.5. Tissue sampling). Animals that became moribund or with markedly disturbed general conditions during the study were euthanized and subjected to macroscopic post mortem examination.

In experiment 2, 268 mice were fed LF or FOS diet spiked with either different doses of single POP congeners ($n=12$) or a mixture of POPs ($n=16$) (Table S2) for 12–14 weeks.

In experiment 3, 96 mice were fed LF or high fat diet based on corn oil with either a high content of sucrose (COS) or protein (COP) ($n=16$) for 16 weeks, and the diets were given with or without a POP mixture (Table S3).

In all the experiments, the mice were weighed once per week, fresh water was provided twice per week, food was changed and the intake was recorded three times per week. Fish oil added to the diets was commercially available cod liver oil (Møllers Tran, Axellus AS, Norway). In all the experimental diets with POPs added, the selected amount of POP congeners (PCB-153, PCB-118 and PCB-138 from Chiron AS, Norway; pp'-DDE from Chem Service, West Chester, USA) were first dissolved in DMSO, next dissolved in part of the oil for the diet and finally added together with the rest of the oil to the respective diets. DMSO was added to the reference diets to a similar concentration

of that of the POP-containing diets, and the amount of DMSO did not exceed 0.9 g/kg feed. The amount of POPs added to the different diets is shown in Tables S2 and S3.

2.2. Determination of POP levels

POPs were measured in the diet from experiment 1 and in liver and adipose tissue of the mice as described [28], including congeners within the group of PCBs and DDTs as shown in Tables S4 and S5.

2.3. Body composition of mice

Whole body composition of fat mass, lean mass and free water mass were determined in live mice by noninvasive scanning using the Bruker Minispec LF50 Body Composition Analyzer mq 7.5 (Bruker Optik GmbH), which uses a time-domain nuclear magnetic resonance system as previously described [29].

2.4. Glucose, insulin and pyruvate tolerance tests

Glucose tolerance test was performed after a 6-h fasting period where mice received 3 mg glucose per gram of lean body mass by oral gavage and after a 16-h fasting period where mice received 1.5 mg glucose per gram of total body mass by oral gavage. Insulin tolerance test was performed by injection of 1 U insulin per kilogram of lean body mass (Actrapid, Denmark) in fed mice. Pyruvate tolerance test was performed by injection of 3 mg sodium pyruvate (Sigma-Aldrich) per gram of lean body mass in 6-h fasted mice. During all tests, blood was collected from the lateral tail vein at the indicated time points and blood glucose was measured using a glucometer (Ascensia Contour, Bayer, Norway).

2.5. Tissue sampling

At termination, the mice were subjected to 4% isoflurane anesthesia (Isoba Vet, Schering-Plough, Denmark). From an uncovered thoracic cavity, blood samples were collected in tubes containing heparin (2 U/ μ l), centrifuged (5 min at 2400g), and plasma was stored at -80°C for further analyses. Adipose tissues and livers were immediately dissected out, weighed, snap-frozen in liquid nitrogen and stored at -80°C .

2.6. Plasma measurements

Insulin levels in plasma were determined using an Insulin (Mouse) ELISA kit (DRG Diagnostics, Germany), and plasma glucose was measured by a glucose assay kit (BioVision, USA). The quantitative insulin sensitivity check index was calculated using the measured plasma levels of insulin and glucose according to the formula $1/[\log(\text{fasting insulin } (\mu\text{U/ml})) + \log(\text{fasting glucose } (\text{mg/dl}))]$.

2.7. Lipid analysis

The composition of lipid classes in liver was quantified using high-performance thin-layer chromatography as previously described [30].

2.8. Quantitative reverse-transcriptase PCR (qRT-PCR)

Total RNA was purified from frozen liver samples, cDNA was synthesized and qRT-PCR was run as previously described [31]. Gene-specific primers for qRT-PCR analyses were designed using Primer Express 2.0 (Applied Biosystems) (primer sequences are available on request). The most stable housekeeping gene, as indicated in the results, was determined using geNorm and further used to normalize the gene expression level of target genes.

2.9. Statistical analysis

All data are presented as mean \pm standard error of the mean (S.E.M.). The variances of all data sets were tested for homogeneity by Levene's test and log-transformed if not homogenous. Statistical differences between several groups were determined by ANOVA using unequal N HSD *post hoc* test and Dunnett's *post hoc* test when only compared to one control group. A factorial ANOVA was used when indicated, with the exposure of POPs and dietary macronutrient composition as categorical predictors to analyze the effect of two different variables (POP exposure or diet), with further unequal N HSD *post hoc* test. Repeated-measures ANOVAs were performed on growth curves. Data with $P < 0.05$ are indicated as significantly different. Statistics were performed using Statistica 12 (StatSoft). For glucose, insulin and pyruvate tolerance tests, the area under the curve was determined by GraphPad Prism 6 (GraphPad Software Inc.), and this software was also used for comparison of survival curves with the log rank (Mantel-Cox) test.

3. Results

3.1. Long-term accumulation of POPs

The protein:sucrose ratio determines the obesogenic effect of a fish-oil-enriched diet [23,24], and the accumulation of POPs depends on whether adipose tissue mass is increasing, stable or decreasing [26,27,32]. Therefore, we fed mice fish-oil-based high fat diets (Table S1) with high sucrose (FOS) or high protein (FOP) content during a long-term feeding trial. The levels of POPs were higher in the fish-oil-based diets than in the LF reference diet (Table S4), and we observed higher content of POPs in adipose tissue of the fish-oil-fed mice after 18 months as compared to 6 months of experimental feeding (Fig. 1A and Table S5). The protein:sucrose ratio of the high fat diets affected the total accumulation of POPs in gonadal white adipose tissue (gWAT), where a high protein intake was associated with reduced accumulation of POPs. Thus, long-term accumulation of POPs from fish oil intake may be influenced by the protein:sucrose ratio. Furthermore, a high protein intake was associated with reduced gWAT mass and body weight gain after 6 and 18 months (Fig. 1B and C). Despite significantly greater adipose tissue mass in FOS-fed mice compared with FOP-fed mice after 18 months of feeding, lifespan as determined by 50% survival seemed unaffected by the protein:sucrose ratio (Fig. 1D), whereas lifespan of both groups was reduced relative to mice fed the LF diet.

3.2. Dose-dependent accumulation of POPs in adipose tissue

Four abundant POPs found in marine sources (PCB-138, PCB-153, PCB-118 and pp'-DDE) reported to be associated with obesity and/or type 2 diabetes [5,7,17,33] were selected and given separately or as a mixture at different doses in the FOS diet (Tables S1–S3). A dose-dependent accumulation in adipose tissue from the mice fed single POPs (Fig. 2) or a mixture of POPs (Fig. 3) was observed. However, despite a wide range of accumulation of POPs in adipose tissue, no effect of either the single congeners or the POP mixtures was observed with respect to fat mass, with the exception of an increase in gWAT mass of mice fed the FOS diet spiked with 1000 µg/kg PCB-153 (Figs. 2 and 3). Further, no differences were observed in feed efficiency or glucose tolerance (Fig. S1). Thus, dose-dependent accumulation of POPs in adipose tissue did not affect obesity development or glucose tolerance.

3.3. Total accumulation of POPs in adipose tissue

To further evaluate the impact of macronutrient composition on adipose tissue accumulation of POPs as a possible contributing factor in obesity development, we included a low-dose POPs mixture, with POP levels comparable to those found in marine sources. As fish oil has previously been demonstrated to protect against obesity development and insulin resistance [34,35] and corn oil constitutes an abundant fat

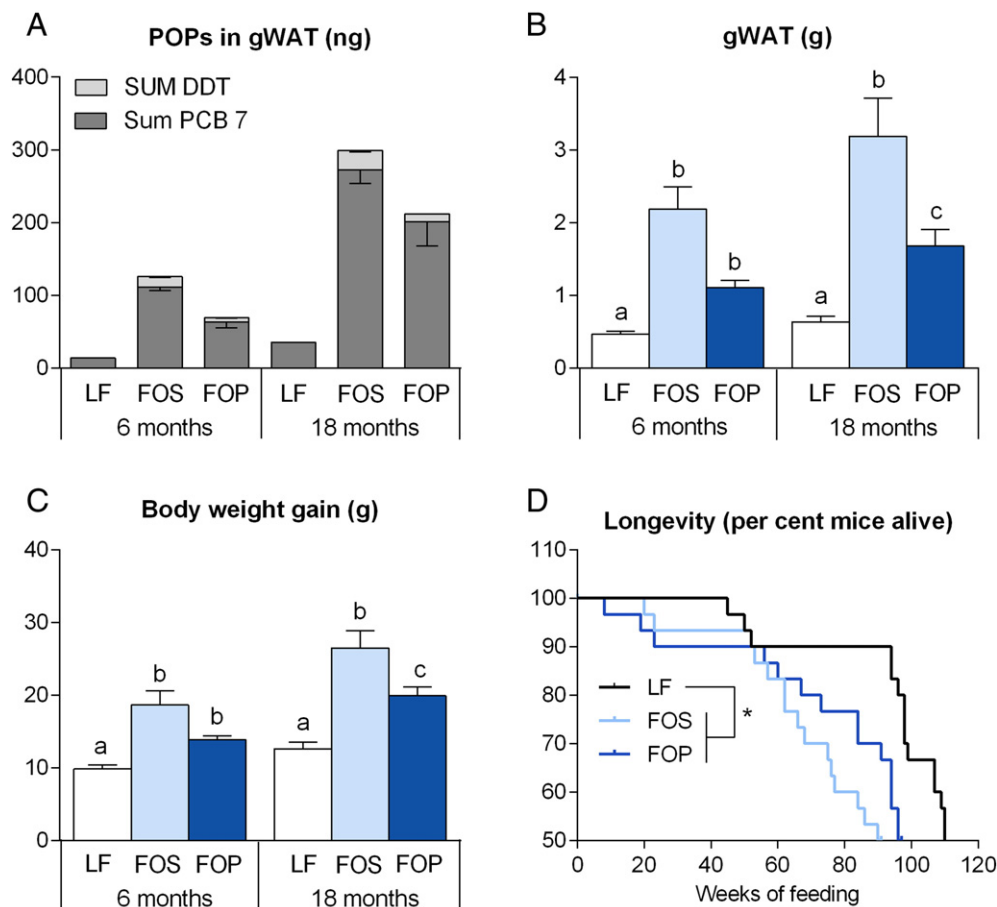


Fig. 1. Long-term accumulation of POPs. (A) Levels of POPs measured in pooled samples of gWAT ($n=3-4$). Detection limit <0.3 µg/kg (<0.2 µg/kg for PCB-118). (B) gWAT mass ($n=9-10$) and (C) body weight gain ($n=9-10$) after 6 and 18 months (mean \pm S.E.M.). (D) Longevity demonstrated as percent mice alive until 50% survival of mice fed LF, fish oil and sucrose (FOS) and fish oil and protein (FOP) diets. *Survival curves of FOS and FOP fed animals were significantly different from mice fed LF, $P<.05$ using log rank (Mantel-Cox) test. Different letters denote significant differences; $P<.05$ by one-way ANOVA using unequal N HSD *post hoc* test.

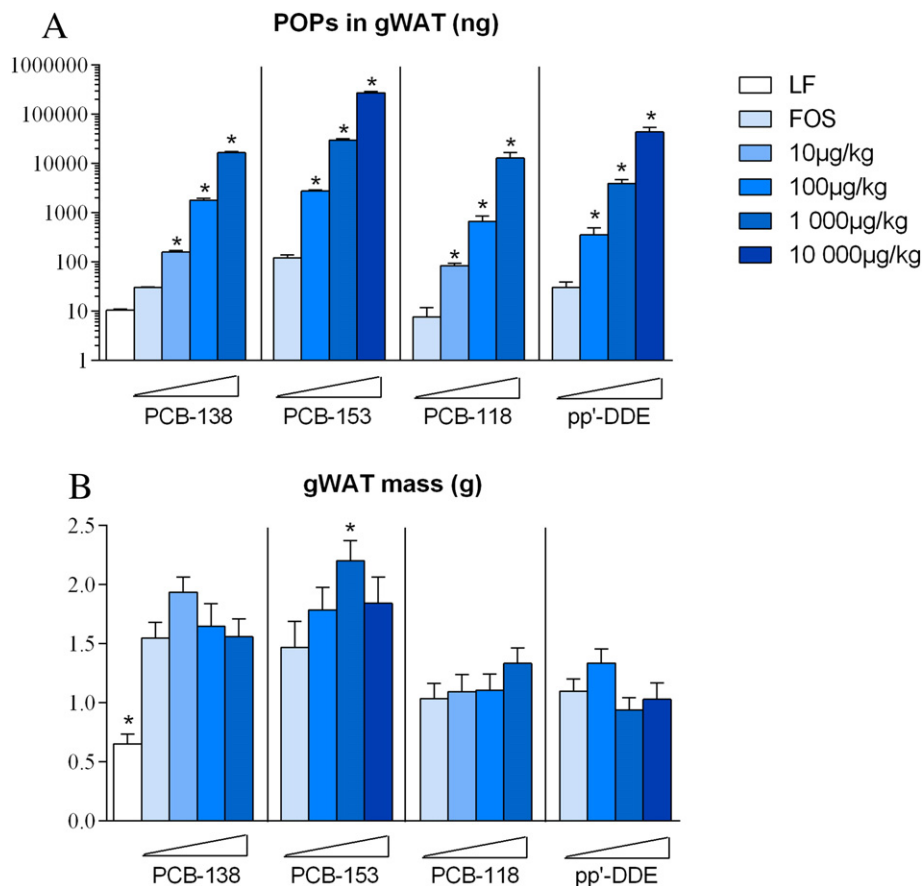


Fig. 2. Dose-dependent accumulation of POPs. (A) POP levels (ng) measured in gWAT ($n=2-6$) and (B) gWAT mass (g) ($n=11-12$) from mice fed LF diet or fish oil and sucrose (FOS) diet spiked with the denoted POP congeners for 12–14 weeks of feeding. To the FOS diet spiked with PCB-138 or PCB-118 was added 0, 10, 100 or 1000 µg/kg of PCB-138 or PCB-118. To the FOS diet spiked with PCB-153 or pp'-DDE was added 0, 100, 1000 or 10,000 µg/kg PCB-153 or pp'-DDE. All data are presented as mean \pm S.E.M. * $P<.05$ compared with FOS diet without added POPs as control group by one-way ANOVA using Dunnett's *post hoc* test.

source in contemporary western diets, we used three different corn-oil-based diets. High fat diets with high content of sucrose (COS) or protein (COP) and a LF diet (Table S1) were given with and without a low-dose POP mixture of the four selected POPs (PCB-138, PCB-153, PCB-118 and pp'-DDE) (Table S3). As expected, all selected POPs accumulated in adipose tissue (Fig. 4A). However, the total accumulation of POPs was dependent on the macronutrient composition and was not directly related to the total intake of POPs. Despite a higher

intake of POPs in the mice fed COP+POP as compared to the mice fed LF+POP ($P=.034$) (data not shown), the mice fed COP+POPs deposited less POPs in the adipose tissue. As predicted, intake of the different diets elicited significant alterations in body weight and fat mass (Fig. 4B–D). The COS-fed mice had increased body mass, fat mass and adipose tissue burden of POPs compared to the COP-fed mice. In contrast, the COP-fed mice had a lower body weight and fat mass as well as a lower adipose burden of POPs compared to COS- and LF-fed

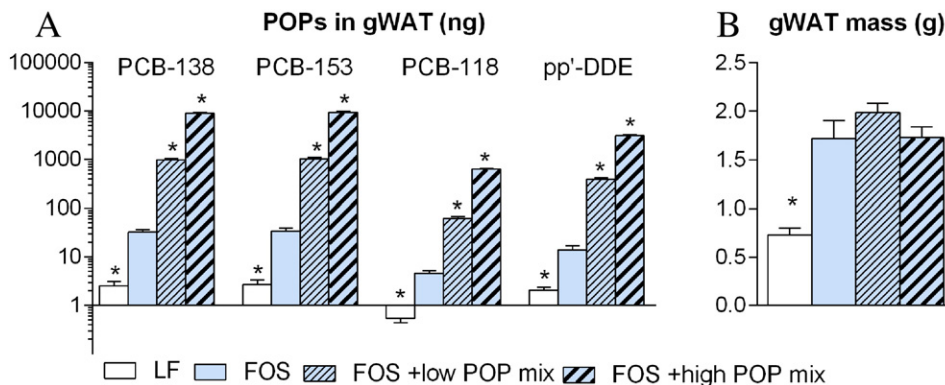


Fig. 3. Dose-dependent accumulation of POPs from mixtures. (A) POP levels (ng) measured in gWAT ($n=4$) and (B) gWAT mass (g) ($n=15-16$) from mice fed LF diet or fish oil and sucrose (FOS) diet spiked with the denoted POP mixtures as shown in Table S2 after 14 weeks of feeding. All data are presented as mean \pm S.E.M. * $P<.05$ compared with FOS diet without added POPs as control group by one-way ANOVA using Dunnett's *post hoc* test.

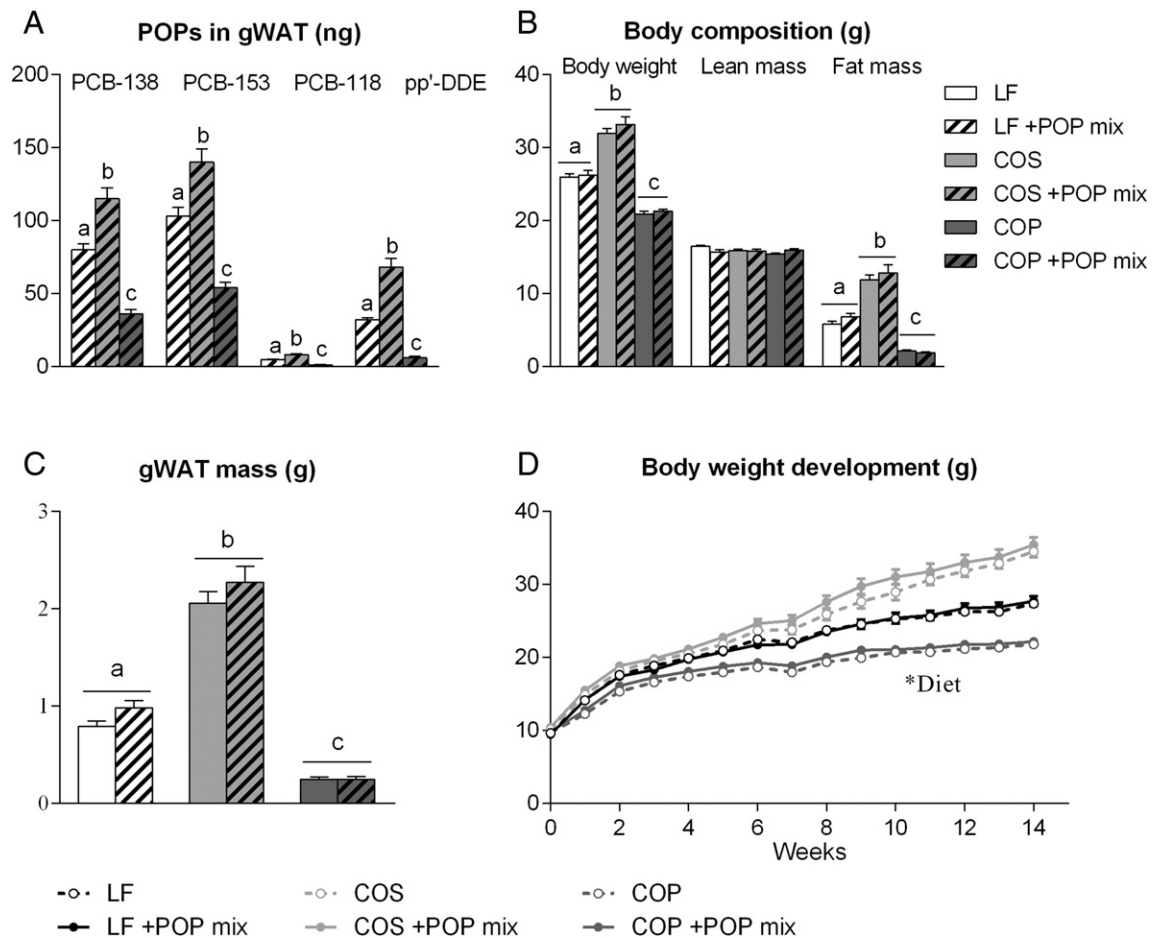


Fig. 4. Accumulation of POPs from different diets. (A) POP levels (ng) measured in gWAT ($n=2-4$) at termination after 16 weeks of feeding. (B) Body composition at week 12, (C) the mass of gWAT at termination and (D) body weight development ($n=16$) during 14 weeks of feeding from mice fed LF, corn oil and sucrose (COS) and corn oil and protein (COP) diets with and without addition of POP mixture as shown in Table S3. All data are presented as mean \pm S.E.M. Different letters denote significant differences between diets; $P < .05$ by factorial ANOVA using unequal N HSD *post hoc* test with dietary macronutrient composition and dietary POPs as categorical predictors. *Diet: $P < .05$ between all diets by repeated-measures ANOVA.

mice. However, within each diet, inclusion of POPs did not alter body or fat mass in mice. Accordingly, total burden of POPs in adipose tissue seems to be determined by the macronutrient composition, but it has no impact on obesity development.

3.4. Glucose tolerance and insulin sensitivity

In addition to insulin, glucose and pyruvate tolerance, plasma levels of glucose and insulin were measured. Levels of glucose in plasma did not differ between the groups in either fed or fasted state (Fig. 5A), but levels of insulin were higher in plasma of mice fed COS compared to the mice fed COP (Fig. 5B). Insulin sensitivity, estimated by insulin tolerance tests (Fig. 5C and D), was reduced in the COS-fed mice as compared to the LF- and COP-fed mice. However, inclusion of the POP mixture in the diets had no impact on insulin sensitivity. Furthermore, in keeping with previous observations [23], intake of the COP diet reduced glucose and pyruvate tolerance (Fig. 5E and F and Fig. S2) possibly related to a higher rate of gluconeogenesis and thereby increased hepatic glucose output. Neither glucose nor pyruvate tolerance was affected by intake of the POP mixture. Thus, in the present study, insulin sensitivity, glucose tolerance and gluconeogenesis did not change with intake of POPs but were highly dependent on the dietary macronutrient composition.

3.5. Hepatic accumulation of POPs, lipid metabolism and detoxification

In the experiments with fish-oil-containing diets, we observed a dose-dependent accumulation of POPs in the livers of mice according to the exposure to POPs, both as single congeners (Fig. S3A) and as a mixture (Fig. S3B). However, the hepatic accumulation of POPs did not affect levels of liver triacylglycerols (TAGs) and total liver lipids in the mice (Fig. S3A and B). Rather, intake of corn oil diets with different macronutrient ratios had great impact on hepatic lipid composition and POP accumulation, where a high protein intake (COP) reduced hepatic accumulation of POPs (with the exception of the PCB-138 congener) (Fig. 6A) and TAGs (Fig. 6B). In addition, the ratio between accumulated POPs and the amount of TAGs in liver was similar in mice fed the LF and COS diets, but an increase in the level of PCBs relative to liver TAGs was observed in response to COP feeding (Fig. 6C). The increase in hepatic PCBs accumulation relative to TAGs indicates that mechanisms apart from a change in lipid accumulation could be involved in the deposition of hepatic PCBs in the COP-fed mice.

Hepatic expression of genes involved in metabolism of xenobiotics was measured, including modification (phase I), conjugation (phase II) and excretion (phase III). Interestingly, we observed that intake of the COP diet in itself led to increased expression of six genes involved in metabolism of xenobiotics, comprising genes involved in phase I (*Cyp1a2*, *Cyp4a14*), phase II (*Sult1a1*, *Gsto1*, *Gstt2*) (Fig. 7) and phase III

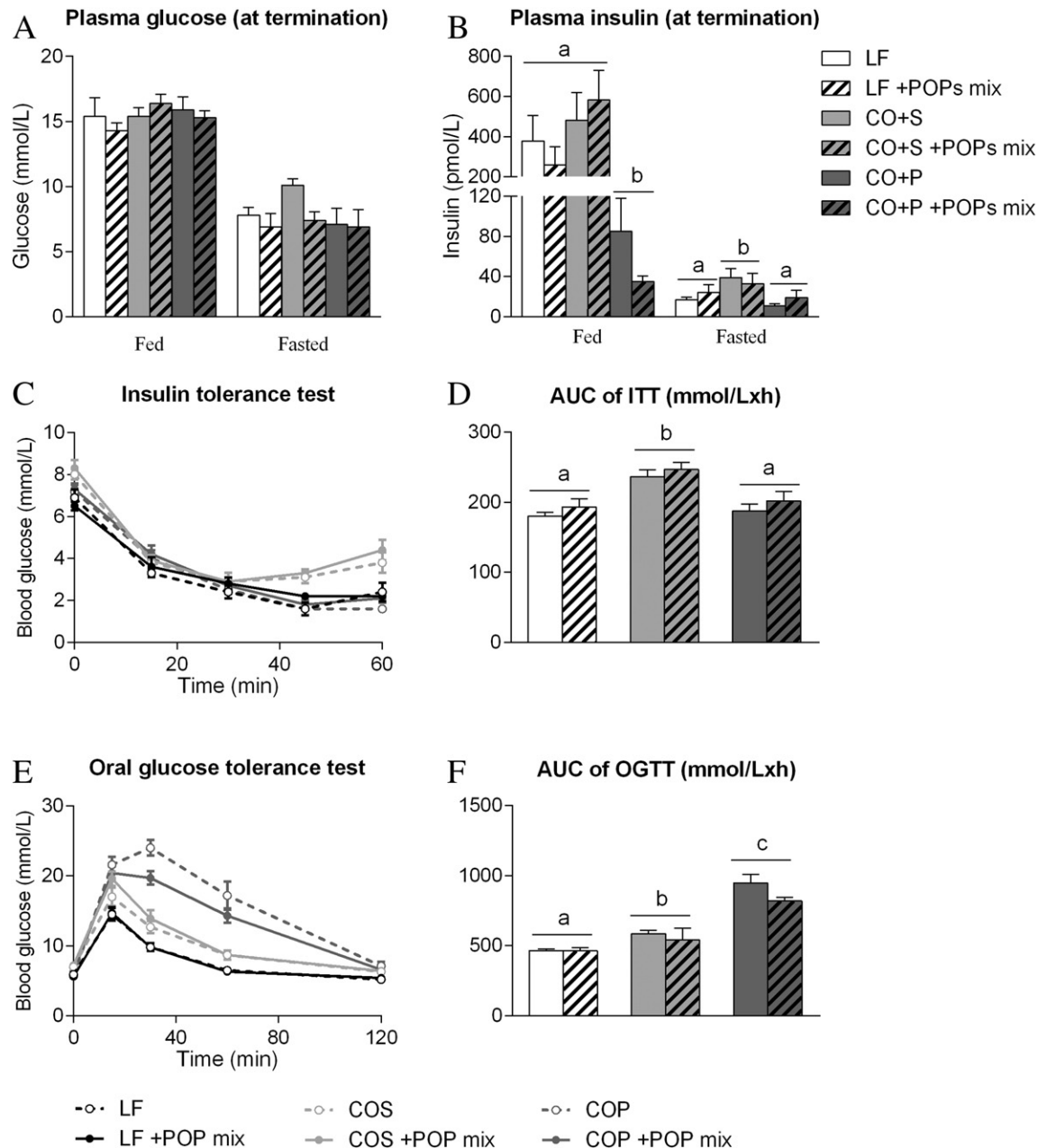


Fig. 5. Glucose tolerance and insulin sensitivity. (A) Fed and 16-h fasted plasma levels of glucose and (B) insulin at termination after 16 weeks of feeding. (C) Insulin tolerance test and (D) area under the curve (AUC) calculated in fed animals (week 11 of feeding). (E) Oral glucose tolerance test and (F) AUC calculated in 6 h fasted animals dosed with 3 mg glucose per gram of lean body mass in week 12 in mice fed LF, corn oil and sucrose (COS) and corn oil and protein (COP) diets both with and without added POP mixture as shown in Table S3. All data are presented as mean \pm S.E.M. Different letters denote significant differences between diets, $P < .05$ by factorial ANOVA using unequal N HSD *post hoc* test with dietary macronutrient composition and dietary POPs as categorical predictors.

(*Abcb1b*) of xenobiotic metabolism (Fig. 8A). In line with altered expression of genes encoding enzymes involved in detoxification and excretion, we also observed changes in the hepatic concentration of phosphatidyl choline (PC) and in the ratio of phosphatidyl ethanolamine (PE) to PC (Fig. 8B and C) that could indicate an increase in bile excretion upon high protein feeding. The POP mixture *per se* had minor effect on hepatic gene expression, but a minor induction of *Cyp4a14* and *Gstt2* mRNA was observed in response to pp'-DDE and PCB-153 exposure (Fig. S4). Together, these findings are in keeping with the observation that only minor alterations in gene expression were detected in response to exposure of the individual POP congeners in the dose-response trials. Thus, inclusion of POPs at concentrations used

in our experiments had a minor effect on a subset of genes involved in clearance of xenobiotics. However, dietary macronutrient composition appeared to be a more powerful driver of hepatic expression of genes involved in metabolism and elimination of xenobiotics. This may, at least in part, explain the lower accumulation of POPs in the COP-fed mice.

4. Discussion

In keeping with previous observations, we found a striking age-dependent accumulation of POPs in adipose tissue. Of note, mice fed the diet with high protein:sucrose ratio accumulated less POPs in

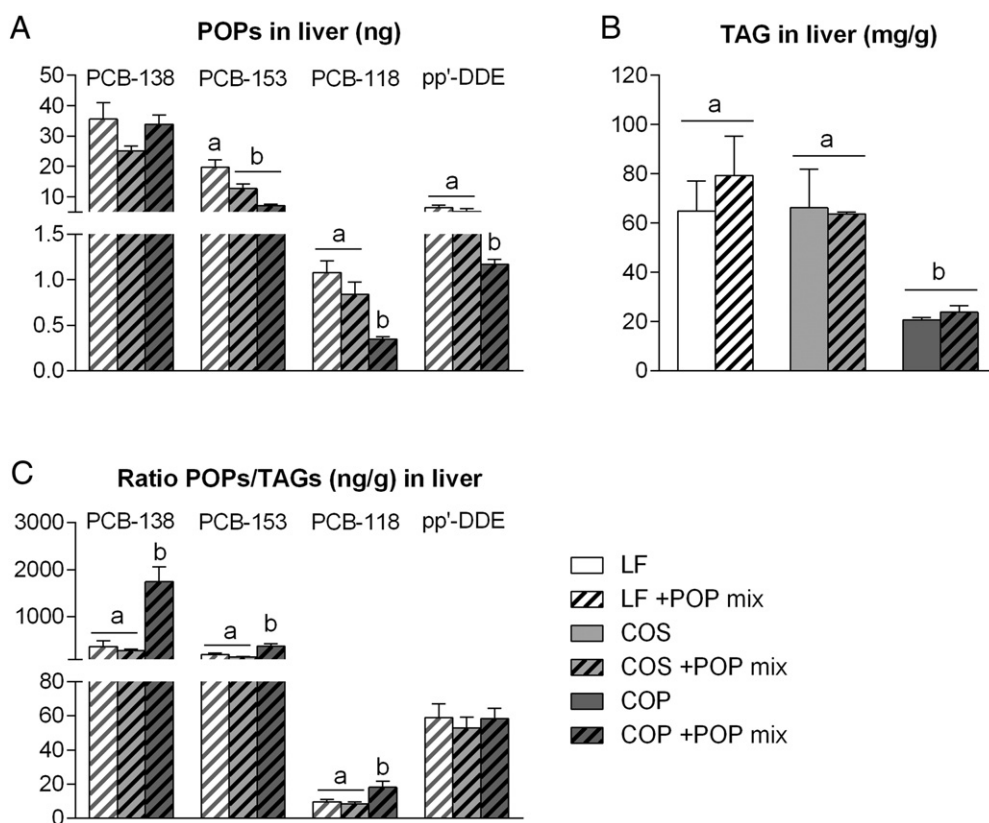


Fig. 6. Hepatic accumulation of POPs and lipids. (A) Hepatic levels of POPs (ng), (B) TAG concentration (mg/g) and (C) amount of POPs (ng) per TAGs (g) ($n=4$) from mice fed LF, corn oil and sucrose (COS) and corn oil and protein (COP) diets with and without added POP mixture as shown in Table S3. All data are presented as mean \pm S.E.M. Different letters denote significant differences between diets; $P<.05$ by factorial ANOVA using unequal N HSD *post hoc* test, with dietary macronutrients and dietary POPs as categorical predictors.

adipose tissue than mice fed diets with low protein:sucrose ratio. The relative abundance of the individual PCBs found in adipose tissue of mice fed fish oil for 18 months in this study was similar to their relative abundance in human adipose tissue [3]. Moreover, the concentrations of the most abundant PCBs found in the adipose tissue from the mice fed fish oil in the present study were within the ranges measured in human adipose tissue in Europe [1,36] and USA [37] but higher than those reported in Asia [3]. In our study, the concentration of the primary metabolite of the pesticide DDT, pp'-DDE, was lower in fish-oil-fed mice than in adipose tissue from humans [1,37,38]. Thus, the concentration found in adipose tissues in the present study can be

considered relevant in the context of normal levels accumulating in humans.

We demonstrate that PCB-118, PCB-138, PCB-153 and pp'-DDE accumulated in adipose tissue in a dose-dependent manner. However, neither in mice fed the fish-oil-based diets nor in mice fed diets supplemented with the pure selected PCBs and pp'-DDE did we observe effects on body weight, glucose tolerance or insulin sensitivity, irrespective of doses or whether the POPs were given as single compounds or in mixtures. The accumulation of POPs in the dose-response experiments ranged from concentrations similar to those found in human adipose tissue and up to 1000-fold higher



Fig. 7. Hepatic expression of genes involved in xenobiotic metabolism. Hepatic expression of *Cyp1a2* and *Cyp4a14* (phase I) and *Sult1a1*, *Gsto1* and *Gstt2* ($n=6-7$) (phase II of detoxification) at termination from mice fed LF, corn oil and sucrose (COS) and corn oil and protein (COP) diets with and without added POP mixture as shown in Table S3. Expression of genes is relative to expression of *Tbp*. All data are presented as mean \pm S.E.M. Different letters denote significant differences between diets; $P<.05$ by factorial ANOVA using unequal N HSD *post hoc* test, with dietary macronutrients and dietary POPs as categorical predictors.

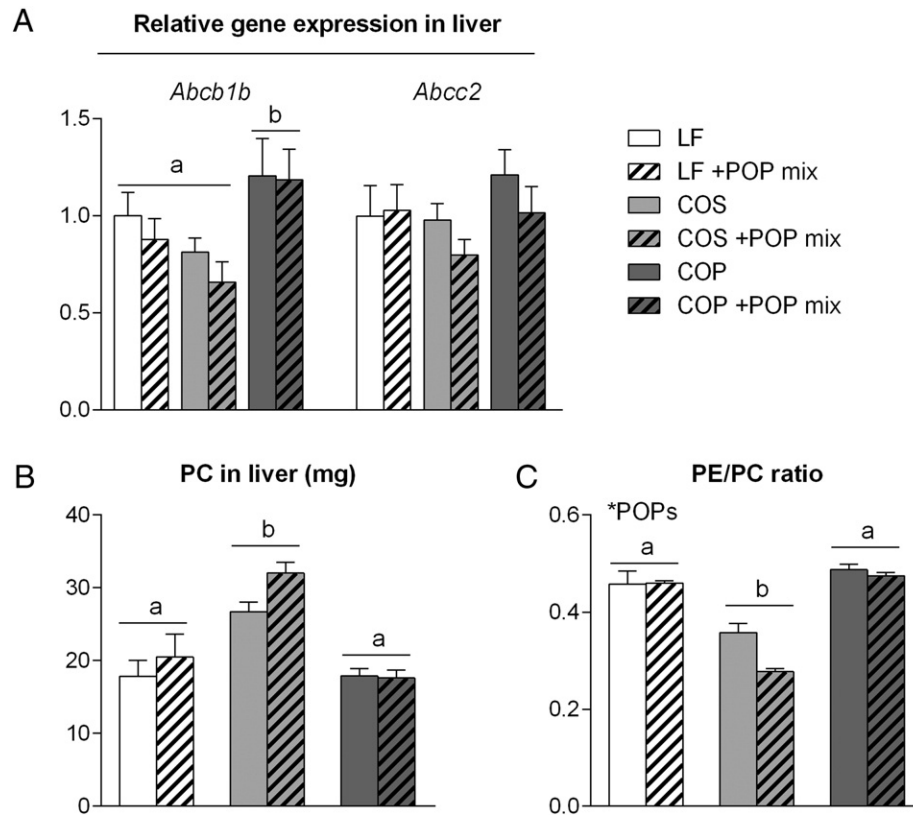


Fig. 8. Gene expression and level of phospholipids in liver. (A) Hepatic expression of genes involved in bile acid transport and phase III of xenobiotic metabolism; *Abcb1b* and *Abcc2* relative to *Tbp* ($n=6-7$). (B) The measured level of PC (mg) in liver and (C) the hepatic ratio of PE per PC ($n=4$). All data are presented as mean \pm S.E.M. Different letters denote significant differences between diets, $P<0.05$; *POPs denotes significant effect of POP exposure, $P<0.05$, by factorial ANOVA using unequal N HSD *post hoc* test, with dietary macronutrients and dietary POPs as categorical predictors.

[1,3,36–38]. In comparison with earlier rodent experiments, where obesity was reported to be affected [17,18], the accumulation of POPs in adipose tissues was similar or more than 100-fold higher.

The relationship between intake of POPs and development of obesity and type 2 diabetes is currently controversial and vigorously debated [10], and conflicting results have also been observed in animal studies [17–20]. Thus, the present study does not support the notion that accumulation of POPs accentuates obesity development and metabolic disorders associated with obesity. However, we cannot exclude the possibility that one or more of the POP congeners and/or POP metabolites present in seafood and other food matrices not investigated here could have changed the outcome on obesity development. In our study, the PCB-118 congener was the only POP congener included with a weak binding affinity to the aryl hydrocarbon receptor AhR, and no coplanar PCB with strong affinity for the AhR was evaluated. A recent study found an effect of coplanar PCBs through the AhR to promote adipose tissue inflammation and reduce glucose tolerance, but only in lean mice or obese mice that lose weight [39], indicating that the background diet and the state of obesity modulate the effects of coplanar PCBs.

We observed that the total burden of POPs in adipose tissue of mice fed diets with a high protein:sucrose ratio was significantly lower than that of mice fed diets with a low protein:sucrose ratio. This phenomenon may be directly related to the well-described ability of high protein diets to attenuate obesity development [23,40]. This interpretation would also be in line with the earlier observation that accumulation of PCBs, at least the highly chlorinated and nearly nonmetabolizable PCB-153, in adipose tissue of rats is depending on adipose tissue expansion [25–27]. In agreement with this, a nearly

linear relationship between body fat and the elimination of POPs has been reported repeatedly [41]. Thus, the positive correlation between POP levels and obesity reported in several studies [7,42,43] may not necessarily indicate that POP exposure is a direct causal factor in obesity development but rather may suggest that a higher body burden of POPs may reflect a higher adipose tissue mass.

We observed an increase in gWAT mass of mice fed FOS diet spiked with 1000 $\mu\text{g/kg}$ PCB-153, but considering the wide range of POPs doses evaluated, the present study provides no evidence that exposure to and accumulation of these POPs affected obesity development, glucose homeostasis or insulin sensitivity. Importantly, the accumulation of selected POPs was dependent on the composition of macronutrients in the background diet. A reduction in body burden of POPs was demonstrated in relation to high dietary protein intake; this was demonstrated for several of the POPs present in fish oil used for the long-term feeding of mice and also for the POPs selected for further evaluation (PCB-118, PCB-153, PCB-138 and pp'-DDE). It is conceivable that accumulation of compounds with similar structure and physicochemical properties would be reduced in response to a high protein diet.

We demonstrate that intake of POPs in a diet with high protein:sucrose ratio caused lower tissue accumulation of POPs than intake of POPs in a diet with low protein:sucrose ratio. Reduced tissue deposition of POPs could either be due to decreased POP absorption from the intestine or it could be caused by a higher elimination from the body. Our hepatic gene expression data indicated that elimination was altered, as genes involved in phases I–III of the detoxifying machinery were induced in mice fed a diet with high protein:sucrose ratio, irrespective of whether POPs were included in the diet or not.

Excretion of POPs through bile would be dependent on bile flow, which is determined by both a bile-acid-dependent fraction and a bile-acid-independent fraction [44]. Different protein sources have been demonstrated to regulate bile metabolism [45,46], and thus, it is possible that dietary protein level can modulate bile flow and, hence, the elimination of POPs. Further studies are required to elucidate the possible role of bile flow in elimination of POPs.

The possible negative effects of environmental pollutants on human health are matters of great concern considering that many of these chemicals are highly resistant to degradation, bioaccumulate in the food web and constitute compounds present in all animal products. Accumulating evidence pointing to possible negative health effects linked to exposure of a wide range of different POPs emphasizes the need to reduce the exposure to POPs. If the dietary composition of macronutrients has an impact on the detoxification and excretion of several POPs, this could potentially be an important and additional way of reducing body burden of POPs and thus reduce the possible detrimental effects of exposure over time.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.jnutbio.2015.09.019>.

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