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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.

Keywords: Macronutrients; Insulin sensitivity; Glucose tolerance; Obesity; Persistent organic pollutants

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/μl), centrifuged (5 min at 2400*g*), 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[fasting insulin (μU/ml)]+log[fasting glucose (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±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 Dunnet'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<.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.