Published in final edited form as:

Toxicol Lett. 2009 September 10; 189(2): 96–101. doi:10.1016/j.toxlet.2009.05.008.

Induction of gene pattern changes associated with dysfunctional lipid metabolism induced by dietary fat and exposure to a persistent organic pollutant

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Abstract

Environmental modulators of chronic diseases can include nutrition, lifestyle, as well as exposure to environmental toxicants such as persistent organic pollutants. A study was designed to explore gene expression changes as affected by both dietary fat and exposure to the polychlorinated biphenyl PCB77. Mice were fed for four months diets enriched with high linoleic acid oils (20 and 40% as calories), and during the last two months half of each group was exposed to PCB77. Ribonucleic acids (RNA) were extracted from liver tissue to determine gene expression changes using DNA microarray analysis. Our microarray data demonstrated a significant interaction between dietary fat and PCB exposure. Deregulated genes were organized into patterns describing the interaction of diet and PCB exposure. Annotation of the deregulated genes matching these probe sets revealed a significant high-fat mediated induction of genes associated with fatty acid metabolism, triacylglycerol synthesis and cholesterol catabolism, which was downregulated in animals exposed to PCB77. Many of these genes are regulated by the peroxisome proliferator activated receptor- α (PPAR- α), and changes in PPAR- α gene expression followed the same gene pattern as described above. These results provide insight into molecular mechanisms of how dietary fat can interact with environmental pollutants to compromise lipid metabolism.

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Conflict of interest statement: The authors have no conflict of interest.

Keywords

PCB77; PPAR α ; nutrition; lipid metabolism; microarray

Introduction

Excessive and uncontrolled hazardous waste sites and the ever increasing use and accumulation of pollutants, and in particular persistent organic pollutants (POPs), are a major environmental and public health concern in the United States. Exposure to environmental chemicals or pollutants can contribute to compromised health and the pathology of many age-related diseases (Hennig et al. 2007b). Human contact to toxic chemicals can originate from environmental and occupational sources and mostly occurs through the food chain, polluted water, as well as through airborne or dermal exposure.

Research over the last decades clearly indicates that the pathology of virtually all age-related or chronic diseases is regulated by multifactorial dietary elements along with other environmental agents and genetic susceptibility (Cordain et al. 2005). Recent studies point to strong associations in humans between serum concentrations of persistent organic pollutants and the pathologies associated with the metabolic syndrome, including liver and cardiovascular disease, and diabetes (Carpenter 2006; Codru et al. 2007; Ha et al. 2007; Lee et al. 2007). Furthermore, in the United States and most developing countries, diet-related chronic diseases represent a major cause of morbidity and mortality. For example, obesity, as a result of overconsumption of high-fat and high-calorie foods and of a sedentary life-style, has become an epidemic and critical risk factor for most chronic and specifically vascular diseases (Poirier et al. 2006).

Many pollutants, such as heavy metals and persistent organics bioaccumulate in our bodies, and bioremediation can become difficult. Furthermore, many environmental pollutants induce signaling pathways that are oxidative stress-sensitive and are similar or the same as the ones associated with the etiology and early pathology of many chronic diseases (Hennig et al. 2005a). Studies derived from epidemiological and basic research and clinical data are evolving which suggest that diet or nutrition, as well as life-style changes, can alter pathologies of chronic diseases as well as diseases associated with environmental toxic insults (Hennig et al. 2007a). For example, in vascular endothelial cells certain dietary fatty acids can amplify inflammatory signaling pathways associated with exposure to POPs such as PCB77 (Hennig et al. 1999), and plant-based nutrients and bioactive compounds such as vitamin E and/or flavonoids can down-regulate PCB-induced vascular inflammation (Hennig et al. 2007b).

Consumption of high fat/high calorie diets can lead to obesity and increased risk for cardiovascular disease or diabetes and exposure to PCBs and other aryl hydrocarbon receptor (AhR) ligands could also increase the risk for these diseases. It is therefore likely that there is a link between environmental risk factors such as nutrition and exposure to environmental pollutants on overall disease outcome (Hennig et al. 2005a). Evolving genomic tools and technologies, including DNA microarray analysis, offer opportunities to gain insight into the cellular response to interactions of nutrition and toxicology on induction of cellular stress and biological mechanisms involved in these biological phenomena (Spielbauer et al. 2005; Gant 2007). DNA microarray technology allows simultaneous measurement of mRNA expression levels from thousands of genes providing a snapshot of the molecular events associated with perturbation of a biological system.

The interaction of nutrition and toxicants and the correlation between these parameters on variation in gene expression is an important and unexplored issue in understanding the

pathology of human diseases. To further explore the paradigm that nutrition can modulate toxicological insults and possibly disease outcome, we designed a study to evaluate the interactive change in gene expression induced by dietary fat and/or exposure to a persistent organic pollutant in mice. Results from our microarray data demonstrate specific gene interaction patterns between dietary fat, PCB exposure, i.e., gene pattern profiles associate mostly with dysfunctional lipid metabolism.

Materials and methods

Animals, diets, and experimental design

Male C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and housed in a pathogen-free environment. Throughout the study, mice were supplied with sufficient food and water. Animal experimental procedures and protocols were approved by the Animal Care and Use Committee (University of Kentucky). For four months mice received two different diets containing linoleic acid (LA) - rich oils (safflower oil; 20 and 40% as calories). The diet composition was similar to the one described earlier (Hennig et al. 2005b), except for the total fat content. Diets were custom prepared and vacuum packed (Dyets Inc., Bethlehem, PA). Diets were based on a modified AIN-76A purified rodent diet (Reeves 1997) with varying levels of safflower oil. During the last two months of the study half of the animals on the two diets were injected intraperitoneally (ip) with PCB77 (170 $\mu\text{mol/kg}$) and the other half received vehicle (safflower oil) injections. Injections were administered every other week. Mice received a total of four injections during this stage of the study. The final injection was administered 48 hours prior to tissue sampling. There were $n=6$ animals per treatment group. This dose regimen was based on previous studies by our group, which suggest that PCB77 exposure can lead to altered lipid metabolism, promote inflammation and atherosclerosis in murine research models (Hennig et al. 2005b; Arsenescu et al. 2008). PCB77 was a generous gift from Dr. Larry Robertson, The University of Iowa, Iowa City, IA. The synthesis and purification method has been published previously (Hennig et al. 1999).

Isolation of mRNA and cDNA synthesis

Total hepatic RNA was extracted using a Qiagen RNeasy Mini Kit (Valencia, CA) according to the manufacturer's directions. RNA concentration was quantified using a Shimadzu UV-1700 Spectrophotometer (Columbia, MD). Complementary DNA (cDNA) was prepared using the Promega Reverse Transcription System (Madison, WI) according to manufacturer's instructions. The RNA used for DNA microarray analysis was obtained from three randomly selected mouse liver tissue samples from each treatment group. The RNA used for real time PCR analysis was extracted from the same tissue samples used for microarray, plus the remaining mouse liver samples from each treatment group.

Microarray analysis

Hepatic RNA samples were prepared from each of 3 randomly selected animals per treatment group and submitted to the University of Kentucky Microarray Facility where gene expression microarray was performed using expression arrays (Affymetrix GeneChips; Santa Clara, CA). Chip normalization and probe set expression measurement were determined using Affymetrix MAS 5. Distributions of gene expression for each chip and correlations between chips revealed no quality control problems. For each probe set, a 2×2 ANOVA model was fit and overall significance of the model was used to determine if there was a significant ($p < 0.01$) difference change in gene expression. This gene list was then sorted using statistical pattern matching in the following way. Pairwise comparisons between PCB-LA20 vs V-LA20, PCB-LA20 vs PCB-LA40, V-LA20 vs V-LA40 and PCB-LA40 vs V-LA40 were recorded as either significantly increased, significantly decreased, or not significantly changed.

Analysis of gene patterns

The constituent genes of each pattern were imported into a web-based bioinformatic search engine, GATHER (gather.genome.duke.edu). GATHER allows the user to functionally annotate a list of genes (Chang et al. 2006). GATHER was used to determine which patterns demonstrated significantly deregulated Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of interest. From the generated patterns, two were chosen for further study because fatty acid metabolism, triacylglycerol synthesis and cholesterol catabolism were significantly deregulated KEGG pathways.

Real time RT-PCR

To validate significant changes in gene expression of a subset of genes (marked in bold in Supplementary Tables 1 and 2) found in deregulated gene patterns of interest among the experimental treatments, quantitative real time PCR was performed with an Applied Biosystems (Foster City, CA) 7300 Real Time PCR System. The TaqMan® Gene Expression Master Mix was used for PCR reactions according to the manufacturer instructions. The thermal cycler conditions were as follows: 55 °C for 2 minutes, 95 °C for 10 minutes, followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 1 minute. Inventoried TaqMan® probes and primers used to quantify mRNA abundance for target genes were purchased from Applied Biosystems. The TaqMan® probes and primers used were: Acadm (assay ID: Mm00431611_m1), Ech1 (assay ID: Mm00469322_m1), Cpt1a (assay ID: Mm00550438_m1), Acot4 (assay ID: Mm00506680_m1), Acs1l (assay ID: Mm00484217_m1), Dgat2 (assay ID: Mm00499530_m1), Agpat6 (assay ID: Mm00497622_m1), Cyp7a1 (assay ID: Mm00484152_m1), PPARα (assay ID: Mm00440939_m1), RXRα (assay ID: Mm00441182_m1), Cyp1a1 (assay ID: Mm00487218_m1) and β-actin (Part No: 4352341E).

Real time PCR and liver-to-body weight ratio statistical analysis

Real time PCR and liver-to-body weight ratio data were analyzed by two way analysis of variance (ANOVA). Post-hoc comparisons of the means were performed by Tukey test. A statistical probability of $P < 0.05$ was considered significant. Statistical analyses for data generated from real time PCR were performed with Sigmapstat (Systat Software, San Jose, CA).

Results

Liver to body weight ratios

Exposure to PCB77 led to increased liver-to-body weight ratios in animals consuming the 40% LA diet (Table 1). Two-way ANOVA, followed by a post hoc comparison, revealed a significant PCB effect in animals receiving the high fat diet. When compared to vehicle treated animals, exposure to PCB77 increased liver to body weight ratio by 114% and 138% in animals consuming the 20 and 40% LA diets, respectively.

Gene expression patterns reveal interaction between diet and PCB exposure

We were interested in exploring the changes in gene expression in the liver of animals exposed to a diet of varying fat content in the presence or absence of an organic pollutant, PCB77. Our microarray data demonstrate a significant interaction between dietary fat and PCB exposure. Approximately 4,000 probe sets were significantly changed due to experimental treatments (diet and/or PCB) and among those probe sets nearly 500 changed significantly due to the interaction of PCB and dietary fat effects. Figures 1A and B depict two gene expression patterns chosen for further study. The pattern represented in Figure 1A, and corresponding Supplementary Table 1, contains 143 deregulated probesets that upon annotation using GATHER revealed a significant high fat (40% LA) mediated induction of genes associated

with fatty acid metabolism. Exposure to PCB77 significantly decreased expression of these genes at both dietary fat levels. The pattern represented in Figure 1B, and corresponding Supplementary Table 2, contains 422 deregulated probesets. These are genes that were significantly induced by the high fat (40% LA) diet and exposure to PCB77 significantly decreased expression of these genes only in animals consuming the high fat (40% LA) diet. Both gene expression patterns had similar characteristics. The number of genes does not match the number of deregulated probesets because of overlap or poor annotation. Genes associated with fatty acid and bile acid metabolism, and triacylglyceride synthesis, were chosen for further exploration by real time PCR. Selected genes are shown in bold in each table.

Verification of real microarray responses

Quantitative real time PCR, using TaqMan probes, was used to validate the results obtained from the microarray analysis on a subset of genes contained in the two patterns depicted in Figures 1A and 1B. Briefly, the high fat diet (40% LA) significantly induced expression of genes associated with triacylglycerol synthesis (Agpat6 and Dgat2; Figure 2A), bile acid metabolism (Cyp7a1; Figure 2B) and mitochondrial and peroxisomal fatty acid metabolism (Acadm, Ech1, and Cpt1a; Figure 2C). Exposure to PCB77 significantly inhibited diet induced effects. Furthermore, PCB77 also decreased basal and diet induced expression of the peroxisome proliferator activated receptor alpha (PPAR α) and the retinoid X receptor alpha (RXR α) (Figure 2D). Overall, the data obtained from real time PCR support the results obtained by DNA microarray analysis.

Gene microarray results indicate that PCB77 treatment significantly induced expression of AHR responsive genes (e.g. CYP1A1 and CYP1A2). PCB77-induced expression of CYP1A1 mRNA was validated by real time PCR (Figure 1E). There was also a trend toward decreased CYP1A1 mRNA levels in high fat (40% LA) animals treated with PCB77 when compared with animals receiving the low fat diet (20% LA) plus PCB77.

Discussion

Disease development can result from both genetic and environmental factors. Environmental factors include lifestyle, nutrition, and exposure to environmental pollutants. Interestingly, metabolic pathways that underlie the pathology of many chronic diseases are similarly influenced or modifiable by both dietary and toxicant factors (Hennig et al. 2007c). Little is known about the paradigm that nutrition can modify toxicological insults and associated diseases. We have reported previously *in vivo* that the type of dietary fat can significantly alter cardiovascular toxicity of PCBs (Hennig et al. 2005b). PCB-induced aortic adhesion molecule (VCAM-1) expression in LDL receptor-deficient mice was markedly enhanced when mice were fed a high-corn oil diet but not a high olive oil diet (Hennig et al. 2005b). In vascular endothelial cells, dioxin-like PCBs can stimulate similar responses as the pro-inflammatory cytokine TNF- α , and we have previously reported that omega-6 fatty acids can amplify PCB-induced endothelial cell toxicity (Hennig et al. 1999). In contrast to omega-6 fatty acids, we recently demonstrated that omega-3 polyunsaturated fatty acids can prevent increases in oxidative stress-sensitive transcription factor activation and adhesion molecule production induced by PCB77 (Wang et al. 2008).

The microarray and real time PCR data obtained from this study strongly suggest that PCB77 exposure disrupts dietary fatty acid induced changes in gene expression. In the liver, as well as in other tissues, unsaturated fatty acids such as linoleic acid act as ligands of PPAR α . Activation of this nuclear receptor pathway leads to increased expression of genes associated with fatty acid and cholesterol metabolism, and triacylglycerol synthesis (Reddy et al. 2001; Patsouris et al. 2006). In our experiment, consumption of a high fat diet increased expression of PPAR α and its DNA binding partner RXR α . The high fat diet also increased mRNA

abundance of PPAR α and RXR α responsive genes associated with peroxisomal and mitochondrial fatty acid metabolism, triacylglycerol synthesis and cholesterol metabolism (Mandard et al. 2004; Lu et al. 2005). Treatment with PCB77 significantly reduced basal and diet induced mRNA expression of PPAR α , RXR α and their responsive genes, such as Acadm, Ech1, Cpt1a, Acsl1, Acot4, Cyp7a1, Dgat2. For example, Agpat6 is a member of the 1-acylglycerol-3-phosphate O-acyltransferase (Agpat) family that appears to be important in triacylglycerol biosynthesis in several tissues (Chen et al. 2008), and this gene appears to be important for triacylglycerol production in mammary epithelium (Beigneux et al. 2006). Another gene that plays a critical role in triacylglycerol synthesis is diacylglycerol O-acyltransferase 2 (Dgat2), which is highly expressed in liver and adipose tissue (Stipanuk 2000; Kohlmeier 2003). We also observed a marked dietary fat/PCB interaction on a gene important in cholesterol/bile acid metabolism (cytochrome P450 7A1). Cyp7a1 was induced by dietary fat and inhibited by PCB77. It has been reported that dietary fatty acids can induce Cyp7a1 expression via activation of PPAR α (Cheema et al. 2000; Kohlmeier 2003).

In addition to the interactive effects of dietary fat and PCB exposure on genes associated with triacylglycerol synthesis and cholesterol/bile acid metabolism, we observed dysfunction of several genes associated with fatty acid metabolism (both synthetic and oxidative pathways), i.e., genes that are also regulated by PPARs. For example, acyl-coenzyme A dehydrogenase-medium chain (Acdm) is a mitochondrial enzyme that catalyzes the initial step in fatty acid metabolism. Acdm expression is increased by PPAR α ligands and disrupted in RXR α deficient animals (Stipanuk 2000; Ringseis et al. 2007). Enoyl coenzyme A hydratase 1 (Ech1) was originally cloned and described from upregulated cDNAs in the livers of rats treated with the PPAR α ligand clofibrate (FitzPatrick et al. 1995). It is a peroxisomal enzyme that participates in the metabolism of long chain fatty acids (Reddy et al. 2001). Carnitine palmitoyltransferase 1a (Cpt1a) plays a critical role in the rate of mitochondrial fatty acid uptake and metabolism (Reddy et al. 2001). Various studies have shown that Cpt1a up-regulation is dependent on PPAR α expression and function (Napal et al. 2005; Tachibana et al. 2006). The dysfunction of some of the genes mentioned above may promote pathologies associated with fatty livers, such as nonalcoholic fatty liver disease (Postic et al. 2008). Overall, our results suggest that dioxin-like PCBs can promote fatty liver disease by inhibiting PPAR α and RXR α expression and function.

Decreased PPAR α and RXR α expression in PCB77 treated animals could be due to PCB-mediated induction of reactive oxygen species production and pro-inflammatory cytokine expression. Previous studies have shown that exposure to dioxin-like PCBs and halogenated aromatic hydrocarbons (HAHs) can promote reactive oxygen species (ROS) production in a variety of tissues (Hassoun et al. 2001; Twaroski et al. 2001; Arzuaga et al. 2006). Increased levels of oxygen radicals have been shown to suppress PPARs α and γ expression and function (Hanlon et al. 2003; El Midaoui et al. 2006; Kim et al. 2007). Cytokine-activated pro-inflammatory cell signaling cascades can also inhibit PPAR function (Zamboni et al. 2006; Kim et al. 2007). In addition to oxidative stress, dioxin-like PCB and HAH induced expression of pro-inflammatory cytokines (Cheon et al. 2007; Arsenescu et al. 2008) could also contribute to compromised PPAR α and RXR α expression in exposed animals. The microarray and real time PCR results obtained from our study support previous findings which demonstrated that exposure to AhR ligands such as PCB77 can negatively alter PPAR α expression and function. *In vivo* and cell culture studies have shown that exposure to AHR ligands can significantly affect PPAR α expression and the induction of PPAR α -responsive genes. For example, AHR activation significantly decreased PPAR α and RXR α protein expression in human hepatocellular carcinoma cells (HepG2) (Shaban et al. 2004). Furthermore, we have shown previously that PCB77 exposure can significantly decrease basal PPAR α and CYP4A1 mRNA expression, as well as PPAR α protein expression in vascular endothelial cells (Arzuaga et al. 2007). Overall, our findings provide an indication of the molecular mechanism supporting the

experimental and epidemiological evidence that exposure to persistent organic pollutants are significant risk factors for hepatic and cardiovascular disease (Chang et al. 2005; Hennig et al. 2005a; Goncharov et al. 2008).

In the current study PCB77 exposure significantly increased liver-to-body weight ratio in animals that consumed the high fat (40% LA) diet. These results validate observations reported by other groups and suggest that a high fat diet can promote hepatotoxic responses to PCBs including oxidative stress, lipid peroxidation and hepatomegaly (Maroni et al. 1981; Twaroski et al. 2001; Fadhel et al. 2002). In addition to regulating fatty acid metabolism in liver tissue, PPAR α has potent anti-inflammatory functions, and its activation has been shown to interfere with pro-inflammatory signaling cascades in various cell types (Zamboni et al. 2006; Zandbergen et al. 2007). Decreased expression and function of PPAR α during hepatic disease states such as alcoholic and non-alcoholic liver disease have been shown to result in fatty acid accumulation and cirrhosis (Crabb et al. 2004; Reddy et al. 2006). Furthermore, PPAR α knockout mice have increased susceptibility to develop fatty liver disease (Costet et al. 1998; Patsouris et al. 2006). Therefore, it is possible that exposure to persistent organic pollutants may promote disease, in part by decreasing PPAR α function and eliminating an endogenous protective mechanism in the liver.

In summary, we provide novel data of gene expression changes associated with the interactions of dietary components and exposure to a persistent organic pollutant. We subsequently identified patterns of gene expression that further supported the contention of interaction of diet and PCB. Our results provide insight into potential mechanisms of how environmental toxicants can interact with or influence hepatic responses to nutritionally relevant fatty acids. Our current findings also support the hypothesis that persistent organic pollutants, and especially pollutants that are ligands of the AhR such as PCB77, are proinflammatory and a risk factor for hepatic and cardiovascular diseases (Hennig et al. 2007b). Finally, our data confirm the paradigm that dietary components, and in this case dietary lipids, can interact with environmental contaminants and thus modify metabolic events associated with liver toxicity and disease (Hennig et al. 2007a).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported in part by grants from NIEHS, NIH (P42ES07380, P20 RR16481), The University of Kentucky Lyman T. Johnson Postdoctoral Fellowship, the University of Kentucky Office of Executive Vice President for Research, and the University of Kentucky Agricultural Experiment Station.

References

- Arsenescu V, Arsenescu RI, King V, Swanson H, Cassis LA. Polychlorinated biphenyl-77 induces adipocyte differentiation and proinflammatory adipokines and promotes obesity and atherosclerosis. *Environ Health Perspect* 2008;116:761–768. [PubMed: 18560532]
- Arzuaga X, Reiterer G, Majkova Z, Kilgore MW, Toborek M, Hennig B. PPAR α Ligands Reduce PCB-Induced Endothelial Activation: Possible Interactions in Inflammation and Atherosclerosis. *Cardiovasc Toxicol* 2007;7:264–272. [PubMed: 17955387]
- Arzuaga X, Wassenberg D, Di Giulio R, Elskus A. The chlorinated AHR ligand 3,3',4,4',5-pentachlorobiphenyl (PCB126) promotes reactive oxygen species (ROS) production during embryonic development in the killifish (*Fundulus heteroclitus*). *Aquat Toxicol* 2006;76:13–23. [PubMed: 16289341]

- Beigneux AP, Vergnes L, Qiao X, Quatela S, Davis R, Watkins SM, Coleman RA, Walzem RL, Philips M, Reue K, Young SG. Agpat6--a novel lipid biosynthetic gene required for triacylglycerol production in mammary epithelium. *J Lipid Res* 2006;47:734–744. [PubMed: 16449762]
- Carpenter DO. Polychlorinated biphenyls (PCBs): routes of exposure and effects on human health. *Rev Environ Health* 2006;21:1–23. [PubMed: 16700427]
- Chang H, Wang YJ, Chang LW, Lin P. A histochemical and pathological study on the interrelationship between TCDD-induced AhR expression, AhR activation, and hepatotoxicity in mice. *J Toxicol Environ Health A* 2005;68:1567–1579. [PubMed: 16076767]
- Chang JT, Nevins JR. GATHER: a systems approach to interpreting genomic signatures. *Bioinformatics* 2006;22:2926–2933. [PubMed: 17000751]
- Cheema SK, Agellon LB. The murine and human cholesterol 7 α -hydroxylase gene promoters are differentially responsive to regulation by fatty acids mediated via peroxisome proliferator-activated receptor α . *J Biol Chem* 2000;275:12530–12536. [PubMed: 10777541]
- Chen YQ, Kuo MS, Li S, Bui HH, Peake DA, Sanders PE, Thibodeaux SJ, Chu S, Qian YW, Zhao Y, Bredt DS, Moller DE, Konrad RJ, Beigneux AP, Young SG, Cao G. AGPAT6 is a novel microsomal glycerol-3-phosphate acyltransferase. *J Biol Chem* 2008;283:10048–10057. [PubMed: 18238778]
- Cheon H, Woo YS, Lee JY, Kim HS, Kim HJ, Cho S, Won NH, Sohn J. Signaling pathway for 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced TNF- α production in differentiated THP-1 human macrophages. *Exp Mol Med* 2007;39:524–534. [PubMed: 17934341]
- Codru N, Schymura MJ, Negoita S, Rej R, Carpenter DO. Diabetes in relation to serum levels of polychlorinated biphenyls and chlorinated pesticides in adult Native Americans. *Environ Health Perspect* 2007;115:1442–1447. [PubMed: 17938733]
- Cordain L, Eaton SB, Sebastian A, Mann N, Lindeberg S, Watkins BA, O'Keefe JH, Brand-Miller J. Origins and evolution of the Western diet: health implications for the 21st century. *Am J Clin Nutr* 2005;81:341–354. [PubMed: 15699220]
- Costet P, Legendre C, More J, Edgar A, Galtier P, Pineau T. Peroxisome proliferator-activated receptor α -isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. *J Biol Chem* 1998;273:29577–29585. [PubMed: 9792666]
- Crabb DW, Galli A, Fischer M, You M. Molecular mechanisms of alcoholic fatty liver: role of peroxisome proliferator-activated receptor α . *Alcohol* 2004;34:35–38. [PubMed: 15670663]
- El Midaoui A, Wu L, Wang R, de Champlain J. Modulation of cardiac and aortic peroxisome proliferator-activated receptor- γ expression by oxidative stress in chronically glucose-fed rats. *Am J Hypertens* 2006;19:407–412. [PubMed: 16580578]
- Fadhel Z, Lu Z, Robertson LW, Glauert HP. Effect of 3,3',4,4'-tetrachlorobiphenyl and 2,2',4,4',5,5'-hexachlorobiphenyl on the induction of hepatic lipid peroxidation and cytochrome P-450 associated enzyme activities in rats. *Toxicology* 2002;175:15–25. [PubMed: 12049832]
- FitzPatrick DR, Germain-Lee E, Valle D. Isolation and characterization of rat and human cDNAs encoding a novel putative peroxisomal enoyl-CoA hydratase. *Genomics* 1995;27:457–466. [PubMed: 7558027]
- Gant TW. Novel and future applications of microarrays in toxicological research. *Expert Opin Drug Metab Toxicol* 2007;3:599–608. [PubMed: 17696809]
- Goncharov A, Haase RF, Santiago-Rivera A, Morse G, McCaffrey RJ, Rej R, Carpenter DO. High serum PCBs are associated with elevation of serum lipids and cardiovascular disease in a Native American population. *Environ Res* 2008;106:226–239. [PubMed: 18054906]
- Ha MH, Lee DH, Jacobs DR. Association between serum concentrations of persistent organic pollutants and self-reported cardiovascular disease prevalence: results from the National Health and Nutrition Examination Survey, 1999–2002. *Environ Health Perspect* 2007;115:1204–1209. [PubMed: 17687448]
- Hanlon PR, Ganem LG, Cho YC, Yamamoto M, Jefcoate CR. AhR- and ERK-dependent pathways function synergistically to mediate 2,3,7,8-tetrachlorodibenzo-p-dioxin suppression of peroxisome proliferator-activated receptor- γ expression and subsequent adipocyte differentiation. *Toxicol Appl Pharmacol* 2003;189:11–27. [PubMed: 12758056]

- Hassoun EA, Li F, Abushaban A, Stohs SJ. Production of superoxide anion, lipid peroxidation and DNA damage in the hepatic and brain tissues of rats after subchronic exposure to mixtures of TCDD and its congeners. *J Appl Toxicol* 2001;21:211–219. [PubMed: 11404832]
- Hennig B, Ettinger AS, Jandacek RJ, Koo S, McClain C, Seifried H, Silverstone A, Watkins B, Suk WA. Using nutrition for intervention and prevention against environmental chemical toxicity and associated diseases. *Environ Health Perspect* 2007a;115:493–495. [PubMed: 17450213]
- Hennig B, Oesterling E, Toborek M. Environmental toxicity, nutrition, and gene interactions in the development of atherosclerosis. *Nutr Metab Cardiovasc Dis* 2007b;17:162–169. [PubMed: 17306736]
- Hennig B, Ormsbee L, Bachas L, Silverstone A, Milner J, Carpenter D, Thompson C, Suk WA. Introductory comments: nutrition, environmental toxins and implications in prevention and intervention of human diseases. *J Nutr Biochem* 2007c;18:161–162. [PubMed: 17296487]
- Hennig B, Reiterer G, Majkova Z, Oesterling E, Meerarani P, Toborek M. Modification of environmental toxicity by nutrients: implications in atherosclerosis. *Cardiovasc Toxicol* 2005a;5:153–160. [PubMed: 16046791]
- Hennig B, Reiterer G, Toborek M, Matveev SV, Daugherty A, Smart E, Robertson LW. Dietary fat interacts with PCBs to induce changes in lipid metabolism in mice deficient in low-density lipoprotein receptor. *Environ Health Perspect* 2005b;113:83–87. [PubMed: 15626652]
- Hennig B, Slim R, Toborek M, Robertson LW. Linoleic acid amplifies polychlorinated biphenyl-mediated dysfunction of endothelial cells. *J Biochem Mol Toxicol* 1999;13:83–91. [PubMed: 9890193]
- Kim MS, Sweeney TR, Shigenaga JK, Chui LG, Moser A, Grunfeld C, Feingold KR. Tumor necrosis factor and interleukin 1 decrease RXRalpha, PPARalpha, PPARgamma, LXRalpha, and the coactivators SRC-1, PGC-1alpha, and PGC-1beta in liver cells. *Metabolism* 2007;56:267–279. [PubMed: 17224343]
- Kohlmeier, M. *Nutrient Metabolism*. Academic Press; New York: 2003.
- Lee DH, Lee IK, Porta M, Steffes M, Jacobs DR Jr. Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999–2002. *Diabetologia* 2007;50:1841–1851. [PubMed: 17624515]
- Lu B, Jiang YJ, Zhou Y, Xu FY, Hatch GM, Choy PC. Cloning and characterization of murine 1-acyl-sn-glycerol 3-phosphate acyltransferases and their regulation by PPARalpha in murine heart. *Biochem J* 2005;385:469–477. [PubMed: 15367102]
- Mandard S, Muller M, Kersten S. Peroxisome proliferator-activated receptor alpha target genes. *Cell Mol Life Sci* 2004;61:393–416. [PubMed: 14999402]
- Maroni M, Colombi A, Arbosti G, Cantoni S, Foa V. Occupational exposure to polychlorinated biphenyls in electrical workers. II. Health effects. *Br J Ind Med* 1981;38:55–60. [PubMed: 6451237]
- Napal L, Marrero PF, Haro D. An intronic peroxisome proliferator-activated receptor-binding sequence mediates fatty acid induction of the human carnitine palmitoyltransferase 1A. *J Mol Biol* 2005;354:751–759. [PubMed: 16271724]
- Patouris D, Reddy JK, Muller M, Kersten S. Peroxisome proliferator-activated receptor alpha mediates the effects of high-fat diet on hepatic gene expression. *Endocrinology* 2006;147:1508–1516. [PubMed: 16357043]
- Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, Eckel RH. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss. *Arterioscler Thromb Vasc Biol* 2006;26:968–976. [PubMed: 16627822]
- Postic C, Girard J. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. *J Clin Invest* 2008;118:829–838. [PubMed: 18317565]
- Reddy JK, Hashimoto T. Peroxisomal beta-oxidation and peroxisome proliferator-activated receptor alpha: an adaptive metabolic system. *Annu Rev Nutr* 2001;21:193–230. [PubMed: 11375435]
- Reddy JK, Rao MS. Lipid metabolism and liver inflammation. II. Fatty liver disease and fatty acid oxidation. *Am J Physiol Gastrointest Liver Physiol* 2006;290:G852–858. [PubMed: 16603729]
- Reeves PG. Components of the AIN-93 diets as improvements in the AIN-76A diet. *J Nutr* 1997;127:838S–841S. [PubMed: 9164249]

- Ringseis R, Gutgesell A, Dathe C, Brandsch C, Eder K. Feeding oxidized fat during pregnancy up-regulates expression of PPARalpha-responsive genes in the liver of rat fetuses. *Lipids Health Dis* 2007;6:6. [PubMed: 17352811]
- Shaban Z, El-Shazly S, Abdelhady S, Fattouh I, Muzandu K, Ishizuka M, Kimura K, Kazusaka A, Fujita S. Down regulation of hepatic PPARalpha function by AhR ligand. *J Vet Med Sci* 2004;66:1377–1386. [PubMed: 15585952]
- Spielbauer B, Stahl F. Impact of microarray technology in nutrition and food research. *Mol Nutr Food Res* 2005;49:908–917. [PubMed: 16189797]
- Stipanuk, MH. *Biochemical and Physiological Aspects of Human Nutrition*. W. B. Saunders Company; Philadelphia: 2000.
- Tachibana K, Anzai N, Ueda C, Katayama T, Kirino T, Takahashi R, Yamasaki D, Ishimoto K, Tanaka T, Hamakubo T, Ueda Y, Arai H, Sakai J, Kodama T, Doi T. Analysis of PPAR alpha function in human kidney cell line using siRNA. *Nucleic Acids Symp Ser (Oxf)* 2006:257–258.
- Twaroski TP, O'Brien ML, Larmonier N, Glauert HP, Robertson LW. Polychlorinated biphenyl-induced effects on metabolic enzymes, AP-1 binding, vitamin E, and oxidative stress in the rat liver. *Toxicol Appl Pharmacol* 2001;171:85–93. [PubMed: 11222084]
- Wang L, Reiterer G, Toborek M, Hennig B. Changing ratios of omega-6 to omega-3 fatty acids can differentially modulate polychlorinated biphenyl toxicity in endothelial cells. *Chem Biol Interact* 2008;172:27–38. [PubMed: 18155686]
- Zambon A, Gervois P, Pauletto P, Fruchart JC, Staels B. Modulation of hepatic inflammatory risk markers of cardiovascular diseases by PPAR-alpha activators: clinical and experimental evidence. *Arterioscler Thromb Vasc Biol* 2006;26:977–986. [PubMed: 16424352]
- Zandbergen F, Plutzky J. PPARalpha in atherosclerosis and inflammation. *Biochim Biophys Acta* 2007;1771:972–982. [PubMed: 17631413]

Figure 1.

Two of the gene expression patterns (A and B) indicate a significant high-fat mediated induction of genes associated with fatty acid metabolism, triacylglycerol synthesis and cholesterol catabolism, which was blocked by PCB77 exposure. For example, a significant difference in deregulated genes matching these probe sets was observed when comparing treatments PCB+LA40 with Vehicle+LA40. Vehicle+LA20 (Vehicle [without PCB] plus 20% fat diet); Vehicle+LA40 (Vehicle plus 40% fat diet); PCB+LA20 (PCB plus 20% fat diet); PCB+LA40 (PCB plus 40% fat diet).

Figure 2.

Real time PCR validation of microarray results for genes associated with A) triacylglycerol synthesis, B) cholesterol metabolism, C) fatty acid metabolism, D) PPAR α and RXR α , and E) AHR activation. Hepatic RNA was extracted from n=6 animals per treatment, including the same animals used for microarray. Bars represent target genes normalized to respective β -actin expression $\pm$ SEM. * and ** represent statistically significant differences ($P < 0.05$) detected by two way ANOVA.

Table 1

Mouse liver to body weight ratios

Treatment	Liver to body weight ratios (g liver weight/g body weight)
20% LA + vehicle	0.0551 ± 0.003
20% LA + PCB77	0.0633 ± 0.001
40% LA + vehicle	0.0489 ± 0.004
40% LA + PCB77	0.0678 ± 0.003 [*]

Results are represented as means ± SEM.

^{*} represent statistically significant differences relative to respective vehicle-treated animals.