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Phytol induced hepatotoxicity in mice

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Abstract

Phytanic acid is a branched-chain, saturated fatty acid present in high concentrations in dairy products and ruminant fat. Some other dietary fats contain lower levels of phytol, which is readily converted to phytanic acid after absorption. Phytanic acid is a peroxisome proliferator binding the nuclear transcription factor peroxisome proliferator-activated receptor α (PPAR α) to induce expression of genes encoding enzymes of fatty acid oxidation in peroxisomes and mitochondria. Administration of dietary phytol (0.5% or 1%) to normal mice for twelve to eighteen days caused consistent PPAR α -mediated responses, such as lower body weights, higher liver weights, peroxisome proliferation, increased catalase expression, and hepatocellular hypertrophy and hyperplasia. Female mice fed 0.5% phytol and male and female mice fed 1% phytol exhibited midzonal hepatocellular necrosis, periportal hepatocellular fatty vacuolation, and corresponding increases in liver levels of the phytol metabolites phytanic acid and pristanic acid. Hepatic expression of sterol carrier protein-x (SCP-x) was five- to twelve-fold lower in female mice than in male mice. These results suggest that phytol may cause selective midzonal hepatocellular necrosis in mice, an uncommon pattern of hepatotoxic injury, and that the greater susceptibility of female mice may reflect a lower capacity to oxidize phytanic acid because of their intrinsically lower hepatic expression of SCP-x.

Keywords

phytol; phytanic acid; PPAR α ; SCP-x; peroxisomes; fatty acids

INTRODUCTION

Phytanic acid is a branched chain, saturated fatty acid present in high concentrations in dairy products and ruminant fat (Steinberg, 1995), serving as the main branched-chain fatty acid in the human diet. Degradation of chlorophyll by ruminal bacteria produces phytol, which is readily converted to phytanic acid in ruminant tissues (Steinberg, 1995). Other dietary fats including vegetable oils contain lower levels of free phytol, which is readily converted to phytanic acid after absorption. There is no endogenous synthesis of either phytol or phytanic acid. Phytanic acid undergoes α - and β -oxidation in peroxisomes, yielding shortened products that are transported to the mitochondria for further oxidation (Reddy and Hashimoto 2001; Seedorf et al. 1998; Westin et al. 2007). In humans, hereditary defects in specific peroxisomal enzymes (for example, Refsum's disease) and generalized peroxisomal disorders cause massive accumulation of phytanic acid and its metabolites (Steinberg 1995; Reddy and Hashimoto 2001; Verhoeven et al. 1998; Wanders et al. 1995). Recent evidence suggests a link

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between high dietary intake of phytanic acid and increased risk of prostate cancer in humans, possibly mediated through the peroxisomal enzyme α -methylacyl-CoA racemase (Mobley et al., 2003, Thornburg et al., 2006).

Phytanic acid stimulates peroxisome proliferation, is the highest affinity naturally occurring ligand that binds the nuclear transcription factor peroxisome proliferators-activated receptor α (PPAR α), and is the most potent naturally occurring ligand that induces expression of genes encoding enzymes of fatty acid oxidation in peroxisomes and mitochondria (Ellinghaus et al. 1999; Gloerich et al. 2005; Gloerich et al. 2007; Hostetler et al. 2006; Reddy and Hashimoto 2001). There is also evidence that phytol as well as phytanic acid can directly bind to and activate PPAR α in intact cells (Goto et al. 2005). Other natural PPAR α activators include very long chain fatty acids, polyunsaturated fatty acids, and eicosanoids (Hostetler et al. 2006; Reddy and Hashimoto 2001). Synthetic PPAR α activators include fibrates, therapeutic hypolipidemic agents (Hostetler et al. 2005). Because of the critical role peroxisomal oxidation plays in the metabolism of branched-chain fatty acids, dietary phytol may be used to investigate the role of specific proteins in peroxisomal uptake and oxidation of lipids. Sterol carrier protein-x (SCP-x) is one such lipid-binding protein which appears to play a key role in the peroxisomal metabolism of branched-chain lipids including phytanic acid. In vitro studies indicate that SCP-x has a dual role, acting not only as a transport protein in the peroxisomal uptake of a variety of lipids, but also as a thiolase enzyme in the oxidation of branched-chain lipids (Seedorf et al. 1998). This paper describes hepatic lesions developing in mice after feeding phytol at two different levels for twelve to eighteen days, and correlation of lesions to liver levels of phytanic acid and its metabolites.

MATERIALS AND METHODS

Animals

Mice in the present study were of a hybrid C57BL/6NCR 129/SvJ background, and were wild type (+/+) littermates from previously described studies (Atshaves et al., 2005, Atshaves et al., 2007). Mice were kept under a twelve-hour light/dark cycle in a temperature-controlled (25° C) facility with access ad libitum to food and water. Mice in the facility were monitored quarterly for infectious diseases and were specific pathogen free, particularly in reference to mouse hepatitis virus. Animal protocols were approved by the Animal Care and Use Committee of Texas A&M University.

Antibodies

Rabbit polyclonal antibody directed against catalase was purchased from Biodesign (Kennebunk, ME, USA). Rabbit polyclonal antibody directed against mouse SCP-x was prepared as described previously (Atshaves et al. 1999). Mouse monoclonal antibody directed against glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was purchased from Millipore (Billerica, MA, USA).

Dietary (phytol) Studies

At seven weeks of age, male and female mice were moved from a standard pelleted rodent chow mix (5% calories from fat) to a modified AIN-76A phytol-free, phytoestrogen-free pelleted rodent diet (5% calories from fat, # D11243, Research Diets, Inc., New Brunswick, NJ, USA) one week before the start of the feeding studies. After one week, one half of the mice remained on the phytol-free diet, whereas the rest were switched to the modified AIN-76A pelleted rodent diet supplemented with either 0.5% or 1.0% phytol (5% calories from fat, Diet # D01020601, Research Diets, Inc.). These amounts are approximately ten to one hundred times the total amount of free phytol and phytanic acid normally present in laboratory rodent diets (Seedorf et al. 1998), though the amount of phytol/phytanic acid in standard laboratory

chow varies widely. Each feeding group consisted of three to eight animals per sex. Four separate studies were undertaken: (a) 0.5% phytol for twelve days, (b) 0.5% phytol for fourteen days; (b) 0.5% phytol for eighteen days; and (c) 1.0% phytol for twelve days. Food intake and mouse body weights were monitored every other day.

Animal Sacrifice and Tissue Collection

At the end of each study, animals were fasted overnight and anesthetized (ketamine 100 mg/kg; zylaxine 10 mg/kg). Whole-body, dual-energy x-ray absorptiometry (DEXA) was performed using a Lunar PIXImus densitometer (Lunar, Madison, WI, USA) to determine the fat tissue mass (FTM) and lean tissue mass (LTM), as previously described (Atshaves et al. 2004; Atshaves et al. 2005). Blood was then collected from the anesthetized mice by cardiac puncture, and the serum was stored at -80°C . Each animal was euthanized by cervical dislocation. Livers were harvested, weighed, and sampled for histological and ultrastructural examination, with the remaining portions snap-frozen on dry ice and stored at -80°C for subsequent analysis.

Histopathology

Liver slices were excised near the porta hepatis and fixed in 10% neutral buffered formalin for twenty-four to forty-eight hours. The tissues were then transferred to 70% ethyl alcohol, processed, and embedded in paraffin. Liver sections (5 μm thick) were stained with hematoxylin and eosin (H&E) for histologic examination under a light microscope. Severity of necrosis was graded as follows: score 0, normal; 1, minimal change; 2, mild change; 3, moderate change; and 4, severe change. Hepatocyte proliferation was assessed by immunohistochemical staining for proliferating cell nuclear antigen (PCNA), using a mouse monoclonal antibody (Clone PC10) (Dako, Carpinteria, CA, USA) and the ARK visualization system (Dako) according to the manufacturer's instructions. Peroxisomes in hepatocytes were stained selectively for catalase using diaminobenzidine (DAB), and peroxisome volume density was determined by morphometric analysis, as described previously (Atshaves et al. 2004).

Western Blotting

Expression of the peroxisomal enzymes catalase and SCP-x was determined by Western blot analysis, as described previously (Atshaves et al. 2004). Briefly, liver homogenates were centrifuged at 100,000 g for one hour to isolate the cytosolic fraction. Cytosolic proteins were separated on tricine gels, transferred to nitrocellulose membranes, and incubated with affinity-purified antibodies against catalase (1:1000 dilution) or SCP-x (1:500 dilution). Proteins were quantified by densitometric analysis, using standard curves generated from Western blots of pure SCP-x. Catalase was expressed as relative intensities. To control for protein loading, Western blots were incubated with an antibody against GAPDH, a common housekeeping gene product, and the expression of catalase and SCP-x was normalized to the mean expression of GAPDH.

Lipid Analyses (phytanic acid and pristanic acid)

Lipid standards were purchased from Nu-Chek Prep, Inc. (Elysian, MN, USA) and Avanti (Alabasta, AL, USA). Phytanic acid and phytol were purchased from Sigma (St. Louis, MO, USA). Pristanic acid was a gift from Dr. Herman J. ten Brink (Free University Hospital, Amsterdam, The Netherlands). Lipids were extracted from liver homogenates with 3:2 (vol/vol) n-hexane-2-propanol and immediately stored under an atmosphere of N_2 to limit oxidation. Fatty acid composition was determined as described previously (Atshaves et al. 2004; Atshaves et al. 2005). The extracted lipid fraction was subjected to acid-catalyzed transesterification followed by extraction into n-hexane and separation by gas-liquid chromatography. Phytol

metabolites (phytanic acid and pristanic acid) were confirmed by gas chromatography-mass spectroscopy.

Statistics

Values were expressed as the mean + SEM. Statistical analysis was performed using the unpaired Student's t test (GraphPad Prism, San Diego, CA, USA). Values with $p < .05$ were considered statistically significant.

RESULTS

There was no significant difference in food consumption between phytol-fed mice and control mice (data not shown). Body weights at the end of the study were significantly lower in male mice on 1% phytol but not 0.5% phytol (Figure 1A). These effects were even more prominent in female mice fed 1% phytol or even 0.5% phytol (Figure 1B). On DEXA scans, the phytol-induced weight loss was a result of reduction of both FTM and LTM (data not shown). Liver weight was significantly increased in most groups of phytol-fed mice, but there was no obvious sex difference (Figures 1C, 1D). Peroxisome proliferation was evident in phytol-fed mice, especially females, as determined by quantitative analysis of Western blots for catalase expression (Figure 2) and light microscopic evaluation of sections stained with DAB for catalase (data not shown).

Grossly, some livers from female mice fed 0.5% phytol and male and female mice fed 1% phytol in the diet were enlarged, pale, and friable, with an accentuated lobular pattern. Microscopically, livers from male mice on 0.5% phytol were usually normal in appearance or exhibited subtle hepatocellular hypertrophy (Figure 3C). In contrast, all female mice on 0.5% phytol and all male and female mice on 1% phytol exhibited necrosis and loss of hepatocytes (Figures 3D–3F), which tended to be most severe in female mice fed 1% phytol (Figure 4). The necrosis was predominantly midzonal in distribution. In some mice, the necrosis extended to involve centrilobular hepatocytes, though there was a tendency to preserve at least the last one or two rows of hepatocytes surrounding the central veins. Based on morphology, cell death appeared to be occurring by both apoptosis (Figure 3D, inset) and non-apoptotic mechanisms (Figure 3F). An inflammatory reaction, consisting mainly of macrophages, with fewer neutrophils in places, was associated with the necrosis in some but not all affected livers. The severity of the inflammation tended to correlate with the severity of the necrosis. In some areas, midzonal apoptosis was occurring with no or minimal associated inflammation. In the most severely affected livers, occasional multinucleated giant cells and foci of mineralization were observed. Additional changes, more pronounced in male and female mice on 1% phytol, included hepatocellular hypertrophy (mainly centrilobular, but occasionally also periportal), and periportal hepatocellular fatty vacuolation. The midzonal necrosis tended to occur at the edge of the zone of periportal hepatocytes undergoing fatty vacuolation (Figures 3E and 3F). Increased numbers of mitotic figures and immunohistochemical staining for PCNA indicated a higher rate of hepatocellular proliferation in phytol-fed mice (Figure 5). Minimal to mild hyperplasia of bile ductule epithelial cells was observed in a minority of phytol-fed mice, but there was no cholestasis, necrosis, or inflammation of the biliary epithelium to suggest injury to the biliary system.

Liver levels of the phytol metabolites phytanic acid and pristanic acid were significantly elevated in female mice on 0.5% phytol and male and female mice on 1% phytol (Figure 6). In female mice, these changes appeared to be time and dose dependent.

Hepatic expression of SCP-x, determined by quantitative analysis of Western blots, was five- to twelve-fold lower in control female mice than in control male mice (Figure 7). Expression

of SCP-x was significantly increased in male mice fed 0.5% phytol and in female mice fed 0.5% phytol and 1% phytol compared with control mice.

DISCUSSION

Phytol-fed mice exhibited consistent PPAR α -mediated responses (Cattley and Popp 2002) such as lower body weights, higher liver weights, peroxisome proliferation, increased catalase expression, centrilobular hepatocellular hypertrophy, and hepatocellular hyperplasia.

A higher dietary phytol level was associated with hepatocellular necrosis and inflammation, presumed to be secondary inflammation. The primarily mid-zonal necrosis appeared to occur by both apoptotic and non-apoptotic mechanisms. Female mice were more susceptible to lower concentrations (0.5%) of dietary phytol than were male mice, as indicated by lower body weights, higher liver weights, and higher necrosis and inflammation scores. At the light microscopic level, there was no evidence of injury to the biliary system, indicating that microscopically visible damage was confined to hepatocytes.

The greater susceptibility of female mice to the toxic effects of dietary phytol may reflect sexual dimorphism in the absorption of phytol from the diet or in any one of numerous metabolic enzymes. One such enzyme is SCP-x, a lipid-binding protein which also catalyzes the thiolase step in the oxidation of branched chain lipids such as phytanic acid (Seedorf et al. 1998). Data from the present and previous studies demonstrate a five-to twelve-fold lower hepatic expression of SCP-x in female mice compared with male mice (Atshaves et al. 2005; Atshaves et al. 2007). Compared with control mice, mice that exhibited hepatic necrosis (female mice on 0.5% phytol and male and female mice on 1% phytol) had significantly higher liver levels of the phytol metabolites phytanic acid and pristanic acid. Both of these metabolites are upstream from the thiolase step catalyzed by SCP-x. Taken together, these results are consistent with female mice having a lower capacity for peroxisomal oxidation of phytanic acid owing to their relatively lower hepatic levels of SCP-x compared to male mice, and provide further in vivo support for the proposed role of SCP-x in branched-chain fatty acid oxidation. These pathology findings confirm and extend a previous observation regarding sexually dimorphic metabolism of phytol in C57BL/6J mice (Atshaves et al. 2004).

The mechanism of hepatocellular necrosis associated with phytanic acid is not known. Hepatocellular damage could potentially result from phytanic acid accumulation leading to hyperstimulation of PPAR α , increased peroxisomal proliferation and enhanced production of hydrogen peroxide (via β -oxidation of pristanic acid in peroxisomes) and accompanying reactive oxygen species, or production of other toxic intermediate compounds such as dicarboxylic acids (via induction of CYP4A and subsequent ω -oxidation of fatty acid in the smooth endoplasmic reticulum). Alternative mechanisms could include interference with mitochondrial function, or membrane damage from insertion of the “thorny” branched-chain phytanic acid molecule in the highly ordered structure of the membrane lipid bilayer (Komen et al. 2007; Steinberg 1995). The necrosis is unlikely to be simply secondary to accumulation of lipid, since in the livers of some female mice fed 0.5% phytol, single-cell necrosis was occurring in the absence of fatty change. Furthermore, in more severely affected livers the necrotic midzonal hepatocytes tended to be at the edge of, rather than within, the zone of periportal hepatocytes that were accumulating lipid. Similarly, a lack of correlation between lipid accumulation and cytotoxicity has been reported from in vitro studies of L cell fibroblasts exposed to phytanic acid (Atshaves et al. 2002).

Midzonal hepatocellular necrosis is unusual, as most hepatotoxins produce centrilobular, and less frequently periportal, necrosis (Cattley and Popp 2002; Kelly 1993). Selective midzonal necrosis has been described with compounds such as ngaione (a furan component from plants

of the genus *Myoporium*) and aflatoxins (Kelly 1993). Contamination of the feed by aflatoxin is considered very unlikely to be the cause of the observed hepatic necrosis, as no lesions were observed in control animals and yet consistent lesions were seen in phytol-fed animals in the four separate studies. It is not clear what specific properties of midzonal hepatocytes predispose them to certain toxins, although it is almost certainly a function of metabolic gradients that exist across the liver lobule, which in some cases may lead to incomplete degradation of reactive intermediate metabolites (Jungermann 1988; Kelly 1993). Susceptibility of midzonal hepatocytes to phytol-induced injury may reflect the net effect of competing, gradient-associated factors including delivery of phytanic acid to the lobules via portal blood, peroxisomal and mitochondrial density, the speed and extent of oxidation of phytanic acid, and production of potentially toxic intermediate compounds including CYP4A metabolites such as dicarboxylic acids.

Abbreviations

GAPDH	glyceraldehydes 3-phosphate dehydrogenase
PCNA	proliferating cell nuclear antigen
PPAR α	Peroxisome proliferator-activated receptor α
SCP-x	Sterol carrier protein-x

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REFERENCES

- Atshaves BP, Petrescu A, Starodub O, Roths J, Kier AB, Schroeder F. Expression and intracellular processing of the 58 kDa sterol carrier protein 2/3-oxoacyl-CoA thiolase in transfected mouse L-cell fibroblasts. *J Lipid Res* 1999;40:610–622. [PubMed: 10191285]
- Atshaves BP, Storey SM, Petrescu A, Greenberg CC, Lyuksyutova OI, Smith R 3rd, Schroeder F. Expression of fatty acid binding proteins inhibits lipid accumulation and alters toxicity in L cell fibroblasts. *Am J Physiol* 2002;283:C688–C703.
- Atshaves BP, Payne HR, McIntosh AL, Tichy SE, Russell D, Kier AB, Schroeder F. Sexually dimorphic metabolism of branched chain lipids in C57BL/6J mice. *J Lipid Res* 2004;45:812–830. [PubMed: 14993239]
- Atshaves BP, McIntosh AL, Payne HR, Mackie J, Kier AB, Schroeder F. Effect of branched-chain fatty acid on lipid dynamics in mice lacking liver fatty acid binding protein gene. *Am J Physiol* 2005;288:C543–C558.
- Atshaves BP, McIntosh AL, Landrock D, Payne HR, Mackie JT, Maeda N, Ball J, Schroeder F, Kier AB. Effect of SCP-x gene ablation on branched-chain fatty acid metabolism. *Am J Physiol* 2007;292:G939–G951.
- Cattley, RC.; Popp, JA. Liver. *Handbook of Toxicologic Pathology*. 2nd ed.. Haschek, WM.; Rousseaux, CG.; Wallig, MA., editors. San Diego, CA: Academic Press; 2002. p. 187-214.
- Ellinghaus P, Wolfrum C, Assman G, Spener F, Seedorf U. Phytanic acid activates the peroxisome proliferator-activated receptor alpha (PPARalpha) in sterol carrier protein 2-/- sterol carrier protein x-deficient mice. *J Biol Chem* 1999;274:2766–2772. [PubMed: 9915808]
- Gloerich J, van Vlies N, Jansen GA, Denis S, Ruiter JPN, van Werkhoven MA, Duran M, Vaz FM, Wanders RJA, Ferdinandusse S. A phytol-enriched diet induces changes in fatty acid metabolism in mice both via PPAR α -dependent and independent pathways. *J Lipid Res* 2005;46:716–726. [PubMed: 15654129]

- Gloerich J, van den Brink DM, Ruiter JPN, van Vlies N, Vaz FM, Wanders RJA, Ferdinandusse S. Metabolism of phytol to phytanic acid in the mouse, and the role of PPAR α in its regulation. *J Lipid Res* 2007;48:77–85. [PubMed: 17015885]
- Goto T, Takahashi N, Kato S, Egawa K, Ebisu S, Moriyama T, Fushiki T, Kawada T. Phytol directly activates peroxisome proliferator activated receptor α (PPAR α) and regulates gene expression involved in lipid metabolism in PPAR α -expressing HepG2 hepatocytes. *Biochem Biophys Res Commun* 2005;337:440–445. [PubMed: 16202384]
- Hostetler HA, Petrescu AD, Kier AB, Schroeder F. Peroxisome proliferator-activated receptor alpha interacts with high affinity and is conformationally responsive to endogenous ligands. *J Biol Chem* 2005;280:18667–18682. [PubMed: 15774422]
- Hostetler HA, Kier AB, Schroeder F. Very-long-chain and branched-chain fatty acyl-CoAs are high affinity ligands for the peroxisome proliferator-activated receptor alpha (PPAR α). *Biochemistry* 2006;45:7669–7681. [PubMed: 16768463]
- Jungermann K. Metabolic zonation of liver parenchyma. *Semin Liv Dis* 1988;8:329–341.
- Kelly, WR. The liver and biliary system. In: Jubb, KVF.; Kennedy, PC.; Palmer, N., editors. *Pathology of Domestic Animals*. 4th ed.. Vol. Vol. 2.. San Diego, CA: Academic Press; 1993. p. 319–406.
- Komen JC, Distelmaier F, Koopman WJ, Wanders RJ, Smeitink J, Willems PH. Phytanic acid impairs mitochondrial respiration through protonophoric action. *Cell Mol Life Sci* 2007;64:3271–3281. [PubMed: 17968498]
- Mobley JA, Leav I, Zielie P, Wotkowitz C, Evans J, Lam YW, L'Esperance BS, Jiang Z, Ho SM. Branched fatty acids in dairy and beef products markedly enhance alpha-methylacyl-CoA racemase expression in prostate cancer cells in vitro. *Cancer Epidemiol Biomarkers Prev* 2003;12:775–783. [PubMed: 12917210]
- Reddy JK, Hashimoto T. Peroxisomal β -oxidation and peroxisome proliferator-activated receptor α : an adaptive metabolic system. *Annu Rev Nutr* 2001;21:193–230. [PubMed: 11375435]
- Seedorf U, Raabe M, Ellinghaus P, Kannenberg F, Fobker M, Engel T, Denis S, Wouters F, Wirtz KWA, Wanders RJA, Maeda N, Assmann G. Defective peroxisomal catabolism of branched fatty acyl coenzyme A in mice lacking the sterol carrier protein-2/sterol carrier protein-x gene function. *Genes Dev* 1998;12:1189–1201. [PubMed: 9553048]
- Steinberg, D. Refsums disease. In: Scriver, CR.; Beaudet, AL.; Sly, WS.; Valle, D., editors. *The Metabolic and Molecular Bases of Inherited Disease*. 7th ed.. Vol. Vol. II.. New York, NY: McGraw-Hill; 1995. p. 2351–2370.
- Thornburg T, Turner AR, Chen YQ, Vitolins M, Chang B, Xu J. Phytanic acid, AMACR and prostate cancer risk. *Future Oncol* 2006;2:213–223. [PubMed: 16563090]
- Verhoeven NM, Wanders RJA, Poll-The BT, Saudubray JM, Jakobs C. The metabolism of phytanic acid and pristanic acid in man: a review. *J Inher Metab Dis* 1998;21:697–728. [PubMed: 9819701]
- Wanders RJA, Schutgens RBH, Barth PG. Peroxisomal disorders: a review. *J Neuropathol Exp Neurol* 1995;54:726–739.
- Westin MAK, Hunt MC, Alexson SEH. Peroxisomes contain a specific phytanoyl-CoA/pristanoyl-CoA thioesterase acting as a novel auxiliary enzyme in α - and β -oxidation of methyl-branched fatty acids in mouse. *J Biol Chem* 2007;282:26707–26716. [PubMed: 17613526]

Figure 1.

Body weight and liver weight. (A, B) Male mice fed 1% phytol and female mice fed 0.5% or 1% phytol have lower body weights than mice fed a control diet. (C, D) Phytol-fed mice have increased liver weights compared with control mice. Data represent means $\pm$ SEM for three to eight mice per group. * $p < 0.05$ vs. control; ** $p < 0.01$ vs. control; *** $p < 0.001$ vs. control.

Figure 2.

Catalase expression in liver determined by Western blot analysis. Phytol-fed mice exhibit increased expression of catalase compared with control mice. Data represent means $\pm$ SEM for three to eight mice per group. * $p < 0.05$ vs. control; ** $p < 0.01$ vs. control; *** $p < 0.001$ vs. control.

Figure 3.

Representative liver sections stained with hematoxylin and eosin. Examples of portal triads (PT) and central veins (CV) are marked. (A) Male mice fed a control diet. (B) Female mice fed a control diet. (C) Liver from a male mouse fed 0.5% phytol exhibits subtle hepatocellular hypertrophy but is otherwise essentially normal. Livers from female mice fed 0.5% phytol (D), male mice fed 1% phytol (E) and female mice fed 1% phytol (F) exhibit necrosis and loss of mid-zonal hepatocytes (arrows), accompanied by inflammation, mild to moderate fatty vacuolation, and hypertrophy of hepatocytes around central veins. (D, inset) Note apoptotic hepatocytes in phytol-fed mice. Bar = 75 μ m.

Figure 4.

Severity grading of hepatocellular necrosis. Male mice fed 1% phytol and female mice fed 0.5% or 1% phytol exhibited necrosis of hepatocytes, which tended to be most severe in female mice fed 1% phytol. Data represent means $\pm$ SEM for three to eight mice per group. * $p < 0.05$ vs. control; ** $p < 0.01$ vs. control; *** $p < 0.001$ vs. control.

Figure 5. Representative liver sections stained immunohistochemically for PCNA. Note increased number of positively stained nuclei in phytol-fed mice (B) compared with control mice (A), indicating increased hepatocellular proliferation. Bar = 75 μ m.

Figure 6.

Phytanic acid and pristanic acid levels in liver. Male mice fed 1% phytol and female mice fed 0.5% or 1% phytol exhibit elevated levels of phytanic acid (A, B) and pristanic acid (C, D) in liver compared with control fed mice. Data represent means $\pm$ SEM for three to eight mice per group. * $p < 0.05$ vs. control; ** $p < 0.01$ vs. control; *** $p < 0.001$ vs. control. ND, not detected.

Figure 7.

Sterol carrier protein-x (SCP-x) expression in liver determined by Western blot analysis.

Female mice fed a control diet have significantly lower levels of SCP-x than male mice fed a control diet. Male mice fed 0.5% phytol and female mice fed 0.5% phytol or 1% phytol exhibit increased expression of SCP-x compared with control mice. Data represent means + SEM for three to eight mice per group. ** $p < .01$ vs. control; *** $p < .001$ vs. control; ## $p < .01$ vs. the respective male control group; ### $p < .001$ vs. the respective male control group.