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Effect of vitamin E on hepatic cell proliferation and apoptosis in mice deficient in the p50 subunit of NF-κB after treatment with phenobarbital

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Abstract

Phenobarbital (PB) is an efficacious and well-studied hepatic tumor promoting agent. Nuclear factor-κB (NF-κB) is a transcription factor activated by reactive oxygen and is involved in cell proliferation and apoptosis. We previously found that PB activates NF-κB and that dietary vitamin E is effective in decreasing PB-induced NF-κB DNA binding. We therefore hypothesized that dietary vitamin E influences PB-induced changes in cell proliferation and apoptosis through its action on NF-κB. NF-κB1 deficient mice (p50^{-/-}) and wild-type B6129 mice were fed a purified diet containing 10 or 250 ppm vitamin E (α-tocopherol acetate) for 28 days. At that time, half of the wild-type and half of the p50^{-/-} mice were placed on the same diet with 0.05% PB for 10 days. Compared to wild-type mice, the p50^{-/-} mice had higher levels of cell proliferation and apoptosis. Cell proliferation was significantly increased by PB, but vitamin E did not affect hepatic cell proliferation. Apoptosis was not changed in mice fed PB, and there was no significant difference in apoptosis between control and high vitamin E treated mice. Thus, vitamin E does not appear to influence cell growth parameters in either wild-type or p50^{-/-} mice.

Keywords

Vitamin E; phenobarbital; NF-κB; cell proliferation; apoptosis

INTRODUCTION

Phenobarbital (PB) is an efficacious and well-studied hepatic tumor promoting agent (Pitot *et al.*, 1987). PB activates the constitutive active/androstane receptor (CAR), which is

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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necessary for the promoting activity of PB (Yamamoto *et al.*, 2004). Possible mechanisms of PB's promoting activity include the inhibition of cell apoptosis, and the induction of cell proliferation, oxidative stress and DNA damage (Butterworth *et al.*, 1995; Schulte-Hermann *et al.*, 1990; Tharappel *et al.*, 2008; Waxman and Azaroff, 1992). The transcription factor NF- κ B, which has been shown to be activated by oxidative stress (Glauert *et al.*, 2008a; Glauert *et al.*, 2008c; Schreck *et al.*, 1992), is critical for the regulation of cell proliferation and apoptosis in the liver (Barkett and Gilmore, 1999; Beg and Baltimore, 1996; Hinz *et al.*, 1999). Previously we observed that PB activates NF- κ B in the liver (Calfee-Mason *et al.*, 2002; Li *et al.*, 1996).

Since NF- κ B can be activated by oxidative stress, one mechanism for inhibiting its activation is by increasing the levels of antioxidants, such as vitamin E, or antioxidant enzymes, such as catalase (Glauert *et al.*, 2008a; Glauert *et al.*, 2008c; Schreck *et al.*, 1992). Vitamin E inhibits the activation of NF- κ B by phenobarbital (Calfee-Mason *et al.*, 2002). Several studies have also shown that vitamin E can inhibit hepatic cell proliferation (Agarwal *et al.*, 2005; Kolaja and Klaunig, 1997; Kolaja *et al.*, 1998) and increase apoptosis (Kolaja and Klaunig, 1997; Kolaja *et al.*, 1998), although the role of vitamin E in liver carcinogenesis is unclear (Glauert *et al.*, 2010).

The objective of this study was to determine if increasing dietary vitamin E would reduce the induction of cell proliferation and the inhibition of apoptosis by PB by reducing the activation of NF- κ B in mice. We tested this hypothesis by feeding mice deficient in the p50 subunit of NF- κ B as well as wild-type mice 10 or 250 mg/kg dietary α -tocopherol acetate for 28 days; half of the mice were then fed 0.05% PB for 10 days. We observed, however, that vitamin E did not influence cell growth parameters in either wild-type or p50 $-/-$ mice.

MATERIALS AND METHODS

Chemicals

Phenobarbital and vitamin E (α -tocopheryl acetate) were obtained from Sigma Chemical Company (St. Louis, MO). Tocopherol stripped corn oil was obtained from Acros Organics (New Jersey). All other constituents of the purified diet were obtained from Teklad Test Diets (Madison, WI). All other chemicals unless otherwise stated were obtained from Sigma Chemical Co. (St. Louis, MO).

Experimental Design

Fifty-six female mice consisting of twenty-four B6129 mice and thirty-two homozygous NF- κ B1 (p50) deficient mice were obtained from our breeding colony (originally purchased from the Jackson Laboratory, Bar Harbor, ME). The mice were split into 8 different groups containing 5–8 mice per group. The mice were housed 3–4 mice/microisolator cage in a temperature- and light-controlled room. The mice were weighed weekly and at the end of the study. The mice were weaned at 3 weeks of age (20–25g) and started on either a high vitamin E (250 ppm) or low vitamin E (10 ppm) purified diet, similar to the AIN-93M diet (Reeves *et al.*, 1993). The composition of the diet was as follows (% of diet): casein (vitamin-free), 14.0; corn starch, 46.57; dextrose monohydrate, 25.50; cellulose fiber, 5.0; corn oil, tocopherol-stripped, 4.0; AIN-93 mineral mix, 3.50; L-cystine, 0.18; choline bitartrate, 0.25; AIN-93M vitamin mix without vitamin E, 1.0. After 28 days on the diets, half of the mice on each diet were administered 0.05% PB in the diet, and were fed the diets for another 10 days. Three days before euthanasia all mice were surgically implanted s.c. with Alzet Osmotic pumps (model 1003D, Durect Corporation, Cupertino, CA) containing 100 μ l of 5-bromo-2'-deoxyuridine (BrdU, 20mg/ml), with a flow rate of 1 μ l/hr for 3 days. Three days after pump implantation the mice were euthanized by overexposure to CO₂ and

the livers were immediately removed and weighed. Three slices of liver were randomly selected from each of the three lobes and were placed in a tissue cassette and stored in 10% buffered neutral formalin (Fisher Scientific SF100-4). The tissues were paraffin embedded and sectioned at 5 μ m and mounted on 3-aminopropyl-triethoxysilane coated glass slides. Tissue sections were used for bromodeoxyuridine (BrdU) immunohistochemical staining or for terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining, as described previously (Calfee-Mason et al., 2008; Glauert et al., 2008b). At least 3000 hepatocellular nuclei were counted randomly per slide to quantify labeling indexes for both cell proliferation and apoptosis.

Statistical Analyses

All statistical analyses were conducted using SYSTAT V.8 (SPSS, Inc.) software. Results were first analyzed by three-way analysis of variance (ANOVA). The results of all ANOVAs are shown in Table 1. If significant interactions were observed, individual differences between means were determined using Bonferroni's posthoc test. The results were reported as means $\pm$ standard error of mean (SEM). The level of significance was $P = 0.05$.

RESULTS AND DISCUSSION

Our objective in this study was to determine if NF- κ B activation is necessary for vitamin E to decrease cell proliferation and increase apoptosis. By measuring cell proliferation and apoptosis in the presence and absence of the NF- κ B p50 subunit, we could determine if vitamin E works through NF- κ B to affect cell proliferation and apoptosis. The final body weights of the wild-type mice were significantly higher than those of the p50 $-/-$ mice (Table 2). Liver weights were decreased in p50 $-/-$ mice, but the liver to body weight ratios were increased in p50 $-/-$ mice, compared with wild-type mice. Phenobarbital treatment did not affect body weight, but the liver weights and liver weight/body weight ratios of PB-treated mice were significantly greater than those of control mice (Table 2). Vitamin E did not significantly affect body or liver weights. Cell proliferation was quantified after a 3 day infusion of BrdU using Alzet osmotic pumps (Figure 1). Cell proliferation was significantly increased by PB. In p50 $-/-$ mice, cell proliferation was increased, but not significantly ($P = 0.08$). Vitamin E did not affect hepatic cell proliferation ($P = 0.30$). Apoptosis was quantified using the TUNEL assay (Figure 2). Representative TUNEL-stained nuclei are shown in Supplemental Figure 1. Apoptosis was not increased in mice fed PB. There was no significant difference in apoptosis between control and high vitamin E treated mice in this study ($P = 0.28$). Hepatic apoptosis was increased in the p50 $-/-$ mice, as has been observed previously (Lu et al., 2004; Tharappel et al., 2003).

This study was designed to test our hypothesis that dietary vitamin E influences PB-induced changes in cell proliferation and apoptosis through its action on NF- κ B. Previously we had shown that dietary vitamin E inhibited the activation of hepatic NF- κ B by PB *in vivo* (Calfee-Mason et al., 2002). Vitamin E also influenced the expression of the NF- κ B-regulated gene I κ B α , but not cyclin D₁ (Calfee-Mason et al., 2008; Calfee-Mason et al., 2004). In the current study, however, vitamin E did not significantly affect hepatocyte cell proliferation. No other studies have examined effects of dietary vitamin E on the induction of cell proliferation by PB. In a study using a similar experimental design, Calfee-Mason et al. (2008) observed that vitamin E inhibited ciprofibrate-induced cell proliferation in p50 $-/-$ mice but not wild type mice. Studies using other agents have observed the inhibition of hepatic cell proliferation by supplemental vitamin E either *in vivo* (Agarwal et al., 2005; Kolaja and Klaunig, 1997; Kolaja et al., 1998) or *in vitro* (Kang et al., 2000; Min et al., 2003). Agarwal et al. (2005) found that supplemental vitamin E inhibited hepatic cell proliferation induced by ferric nitrilotriacetate. Kolaja and Klaunig (1997) observed that

vitamin E deficiency increased cell proliferation in mice treated earlier with diethylnitrosamine but that supplementation above recommended levels had no significant effect. In addition, they observed that both supplementation and deficiency of vitamin E enhanced the growth of altered hepatic foci. Kolaja et al. (1998) subsequently observed that vitamin E inhibited dieldrin-induced cell proliferation in both normal and focal hepatocytes, but supplemental vitamin E alone increased cell proliferation in altered hepatic foci. The reasons for the differences between the present study and other published studies are not known, but could be related to the different chemicals used to induce hepatocyte proliferation or to the different animal models used.

Vitamin E also did not significantly affect hepatocyte apoptosis. No other studies have examined the modification of PB-influenced apoptosis by dietary vitamin E. Using a similar experimental design as the present study, Calfee-Mason et al. (2008) observed that dietary vitamin E increased apoptosis in both ciprofibrate-treated and untreated mice, and in both p50 $-/-$ mice and wild type mice. In the present study, the apoptotic levels in PB-treated mice were slightly increased by feeding higher levels of vitamin E, but neither the vitamin E main effect ($P = 0.28$) nor the vitamin E-PB interaction ($P = 0.20$) reached statistical significance. Kolaja and Klaunig (1997), however, observed that vitamin E deficiency increased hepatocyte apoptosis in mice treated earlier with diethylnitrosamine, but that supplementation decreased apoptosis. Kolaja et al. (1998) subsequently observed that vitamin E inhibited apoptosis in both normal hepatocytes in both dieldrin-treated and untreated mice, but that vitamin E did not affect apoptosis in altered hepatic foci. In *in vitro* studies, tocopherols were also found to inhibit apoptosis at 50 μM in primary human hepatocytes (Gonzalez et al., 2007), at 50 μM in rat 5123tc hepatoma cells (Pandey et al., 2003), and at 1000 μM in the hepatoma-derived M38 cell line (Schmitz et al., 2004). Since normal plasma concentrations are approximately 20 μM (Traber, 2006), the Gonzalez et al. and Pandey et al. studies used concentrations close to the physiological range. Several studies, however, found that tocotrienols induced apoptosis in cultured liver cell lines (Har and Keong, 2005; Sakai et al., 2006; Sakai et al., 2004; Wada et al., 2005). In human hepatitis B patients, low serum vitamin E concentrations were correlated with higher levels of hepatocyte apoptosis (Fan et al., 2009).

CONCLUSIONS

In summary, vitamin E does not appear to influence PB-induced cell growth parameters in either wild-type or p50 $-/-$ mice. Since vitamin E has been found to inhibit PB-induced NF- κB activation, it is likely that there are other PB-induced changes in gene expression that are influenced by vitamin E's inhibition of NF- κB activation. Although both PB administration and the deletion of the p50 subunit independently influenced cell proliferation, the presence of the p50 subunit does not appear to be necessary for the induction of liver cell proliferation by PB. If NF- κB were necessary for these changes, one would expect to observe that PB would induce cell proliferation only in wild-type mice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

ANOVA	analysis of variance
BrdU	5-bromo-2'-deoxyuridine
CAR	constitutive active/androstane receptor
PCB-153	2,2',4,4',5,5'-hexachlorobiphenyl
NF-κB	nuclear factor-κB
p50^{-/-}	NF-κB1 deficient mice
PB	phenobarbital
SEM	standard error of mean
TUNEL	terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling

References

- Agarwal MK, Iqbal M, Athar M. Vitamin E inhibits hepatic oxidative stress, toxicity and hyperproliferation in rats treated with the renal carcinogen ferric nitrilotriacetate. *Redox Rep.* 2005; 10:62–70. [PubMed: 15949125]
- Barkett M, Gilmore TD. Control of apoptosis by Rel/NF-kappaB transcription factors. *Oncogene.* 1999; 18:6910–6924. [PubMed: 10602466]
- Beg AA, Baltimore D. An essential role for NF-kappa B in preventing TNF-alpha-induced cell death. *Science.* 1996; 274:782–784. [PubMed: 8864118]
- Butterworth BE, Conolly RB, Morgan KT. A strategy for establishing mode of action of chemical carcinogens as a guide for approaches to risk assessments. *Cancer Lett.* 1995; 93:129–146. [PubMed: 7600540]
- Calfee-Mason KG, Lee EY, Spear BT, Glauert HP. Role of the p50 subunit of NF-kappaB in vitamin E-induced changes in mice treated with the peroxisome proliferator, ciprofibrate. *Food Chem Toxicol.* 2008; 46:2062–2073. [PubMed: 18336980]
- Calfee-Mason KG, Spear BT, Glauert HP. Vitamin E inhibits hepatic NF-kappa B activation in rats administered the hepatic tumor promoter, phenobarbital. *J Nutr.* 2002; 132:3178–3185. [PubMed: 12368415]
- Calfee-Mason KG, Spear BT, Glauert HP. Effects of vitamin E on the NF-kB pathway in rats treated with the peroxisome proliferator, ciprofibrate. *Toxicol Appl Pharmacol.* 2004; 199:1–9. [PubMed: 15289085]
- Fan XP, Wang K, Liu Y, Wang JF. Plasma alpha-tocopherol is negatively correlated with hepatocyte apoptosis in chronic hepatitis B patients. *Intern Med.* 2009; 48:1585–1593. [PubMed: 19755759]
- Glauert HP, Calfee-Mason K, Li Y, Nilakantan V, Twaroski ML, Tharappel J, Spear BT. The role of NF-kappaB in PPARalpha-mediated hepatocarcinogenesis. *PPAR Res.* 2008a; 2008:286249. [PubMed: 19197377]
- Glauert HP, Calfee-Mason K, Stemm DN, Tharappel JC, Spear BT. Dietary antioxidants in the prevention of hepatocarcinogenesis: a review. *Mol Nutr Food Res.* 2010; 54:875–896. [PubMed: 20512789]
- Glauert HP, Tharappel JC, Banerjee S, Chan LS, Kania-Korwel I, Lehmler HJ, Lee EY, Robertson LW, Spear BT. Inhibition of the promotion of hepatocarcinogenesis by 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153) by the deletion of the p50 subunit of NF-kB in mice. *Toxicol Appl Pharmacol.* 2008b; 232:302–308. [PubMed: 18644402]
- Glauert HP, Tharappel JC, Lu Z, Stemm D, Banerjee S, Chan LS, Lee EY, Lehmler HJ, Robertson LW, Spear BT. Role of oxidative stress in the promoting activities of PCBs. *Environ Toxicol Pharmacol.* 2008c; 25:247–250. [PubMed: 19122744]

- Gonzalez R, Collado JA, Nell S, Briceno J, Tamayo MJ, Fraga E, Bernardos A, Lopez-Cillero P, Pascussi JM, Rufian S, Vilarem MJ, De la Mata M, Brigelius-Flohe R, Maurel P, Muntane J. Cytoprotective properties of alpha-tocopherol are related to gene regulation in cultured D-galactosamine-treated human hepatocytes. *Free Radic Biol Med*. 2007; 43:1439–1452. [PubMed: 17936189]
- Har CH, Keong CK. Effects of tocotrienols on cell viability and apoptosis in normal murine liver cells (BNL CL. 2) and liver cancer cells (BNL 1ME A.7R.1), in vitro. *Asia Pac J Clin Nutr*. 2005; 14:374–380. [PubMed: 16326644]
- Hinz M, Krappmann D, Eichten A, Heder A, Scheidereit C, Strauss M. NF-kappaB function in growth control: regulation of cyclin D1 expression and G0/G1-to-S-phase transition. *Mol Cell Biol*. 1999; 19:2690–2698. [PubMed: 10082535]
- Kang JH, Shi YM, Zheng RL. Effects of ascorbic acid and DL-alpha-tocopherol on human hepatoma cell proliferation and redifferentiation. *Acta Pharmacol Sin*. 2000; 21:348–352. [PubMed: 11324465]
- Kolaja KL, Klaunig JE. Vitamin E modulation of hepatic focal lesion growth in mice. *Toxicology and Applied Pharmacology*. 1997; 143:380–387. [PubMed: 9144454]
- Kolaja KL, Xu Y, Walborg EF, Stevenson DE, Klaunig JE. Vitamin E modulation of dieldrin-induced hepatic focal lesion growth in mice. *J Toxicol Environ Health Pt A*. 1998; 53:479–492.
- Li Y, Leung LK, Spear BT, Glauert HP. Activation of hepatic NF-kB by phenobarbital in rats. *Biochemical and Biophysical Research Communications*. 1996; 229:982–989. [PubMed: 8955003]
- Lu Z, Lee EY, Robertson LW, Glauert HP, Spear BT. Effect of 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153) on hepatocyte proliferation and apoptosis in mice deficient in the p50 subunit of the transcription factor NF-kB. *Toxicol Sci*. 2004; 81:35–42. [PubMed: 15201435]
- Min J, Guo J, Zhao F, Cai D. Effect of different vitamin E homologous analogues on human hepatoma cell HepG2 proliferation in vitro. *Wei Sheng Yan Jiu*. 2003; 32:40–43. [PubMed: 12731285]
- Pandey S, Lopez C, Jammu A. Oxidative stress and activation of proteasome protease during serum deprivation-induced apoptosis in rat hepatoma cells; inhibition of cell death by melatonin. *Apoptosis*. 2003; 8:497–508. [PubMed: 14601556]
- Pitot HC, Goldsworthy TL, Moran S, Kennan W, Glauert HP, Maronpot RR, Campbell HA. A method to quantitate the relative initiating and promoting potencies of hepatocarcinogenic agents in their dose-response relationships to altered hepatic foci. *Carcinogenesis*. 1987; 8:1491–1499. [PubMed: 2888545]
- Reeves PG, Nielsen FH, Fahey GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A diet. *J Nutr*. 1993; 123:1939–1951. [PubMed: 8229312]
- Sakai M, Okabe M, Tachibana H, Yamada K. Apoptosis induction by gamma-tocotrienol in human hepatoma Hep3B cells. *J Nutr Biochem*. 2006; 17:672–676. [PubMed: 16517139]
- Sakai M, Okabe M, Yamasaki M, Tachibana H, Yamada K. Induction of apoptosis by tocotrienol in rat hepatoma dRLh-84 cells. *Anticancer Research*. 2004; 24:1683–1688. [PubMed: 15274341]
- Schmitz EK, Kraus DM, Bulla GA. Tissue-specificity of apoptosis in hepatoma-derived cell lines. *Apoptosis*. 2004; 9:369–375. [PubMed: 15258469]
- Schreck R, Albermann K, Baeuerle PA. Nuclear factor kappa B: an oxidative stress-responsive transcription factor of eukaryotic cells (a review). *Free Radic Res Commun*. 1992; 17:221–237. [PubMed: 1473734]
- Schulte-Hermann R, Timmermann-Trosiener I, Barthel G, Bursch W. DNA synthesis, apoptosis, and phenotypic expression as determinants of growth of altered foci in rat liver during phenobarbital promotion. *Cancer Res*. 1990; 50:5127–5135. [PubMed: 2379175]
- Tharappel JC, Nalca A, Owens AB, Ghabrial L, Konz EC, Glauert HP, Spear BT. Cell proliferation and apoptosis are altered in mice deficient in the NF-kappaB p50 subunit after treatment with the peroxisome proliferator ciprofibrate. *Toxicol Sci*. 2003; 75:300–308. [PubMed: 12883078]
- Tharappel JC, Spear BT, Glauert HP. Effect of phenobarbital on hepatic cell proliferation and apoptosis in mice deficient in the p50 subunit of NF-kappaB. *Toxicol Appl Pharmacol*. 2008; 226:338–344. [PubMed: 17963809]

- Traber, MG. Vitamin E. In: Shils, ME.; Shike, M.; Ross, CA.; Caballero, B.; Cousins, R., editors. Modern Nutrition in Health and Disease. 10. Lippincott Williams & Wilkins; Philadelphia: 2006. p. 396-411.
- Wada S, Satomi Y, Murakoshi M, Noguchi N, Yoshikawa T, Nishino H. Tumor suppressive effects of tocotrienol in vivo and in vitro. *Cancer Lett.* 2005; 229:181–191. [PubMed: 16098658]
- Waxman DJ, Azaroff L. Phenobarbital induction of cytochrome P-450 gene expression. *Biochem J.* 1992; 281 (Pt 3):577–592. [PubMed: 1536639]
- Yamamoto Y, Moore R, Goldsworthy TL, Negishi M, Maronpot RR. The orphan nuclear receptor constitutive active/androstane receptor is essential for liver tumor promotion by phenobarbital in mice. *Cancer Res.* 2004; 64:7197–7200. [PubMed: 15492232]

Research Highlights

- Is NF- κ B activation necessary for vitamin E to alter phenobarbital-induced changes in cell proliferation and apoptosis?
- Dietary vitamin E did not significantly affect hepatocyte cell proliferation or apoptosis
- Phenobarbital administration and NF- κ B1 deletion independently increased hepatocyte cell proliferation
- NF- κ B1 deletion but not phenobarbital administration increased hepatocyte apoptosis

Figure 1. Effect of phenobarbital (PB), vitamin E (VE), and genotype on hepatocyte cell proliferation. The labeling index was determined by BrdU immunohistochemical staining. Values represent mean $\pm$ SEM. *Values are significantly different from their corresponding untreated controls, based on ANOVA. #Values are different from the p50+/+ group that received the same treatment ($p = 0.08$), based on ANOVA.

Figure 2.

Effect of phenobarbital (PB), vitamin E (VE), and genotype on hepatocyte apoptosis. Values represent mean $\pm$ SEM. *Values are significantly different from the p50+/+ mice, based on ANOVA.

Table 1

Results of Three-Way ANOVA for the Study Endpoints (P values)

Study Endpoint	Main effect for vitamin E	Main effect for PB	Main effect for p50 genotype	Vitamin E/PB interaction	Vitamin E/p50 interaction	PB/p50 interaction	Three-way interaction
Body weight	0.90	0.64	<0.001	0.83	0.48	0.61	0.17
Liver weight	0.89	<0.001	0.04	0.79	0.72	0.27	0.26
Liver weight/body weight	0.97	<0.001	<0.001	0.96	0.49	0.99	0.96
Cell proliferation	0.30	0.003	0.08	0.33	0.57	0.33	0.77
Apoptosis	0.28	0.71	0.05	0.20	0.41	0.40	0.29

Table 2
Effect of Genotype, Vitamin E, and Phenobarbital (PB) Body and Liver Weights

Mouse	Dietary Vitamin E	Treatment	Mice per Group	Body weight (g)	Liver weight (g)	Liver weight/body weight (%)
Wild-type	10 mg/kg	Control	5	27.74 ± 2.97	1.21 ± 0.10	4.39 ± 0.31
		PB	6	26.67 ± 2.34	1.90 ± 0.10 ^a	7.19 ± 0.79 ^a
	250 mg/kg	Control	6	26.17 ± 4.17	1.17 ± 0.18	4.51 ± 0.49
		PB	6	27.17 ± 3.19	1.99 ± 0.25 ^a	7.33 ± 0.28 ^a
p50 ^{-/-}	10 mg/kg	Control	8	20.72 ± 0.74 ^b	1.13 ± 0.12 ^b	5.44 ± 0.46 ^b
		PB	8	22.09 ± 1.67 ^b	1.81 ± 0.21 ^{ab}	8.25 ± 0.92 ^{ab}
	250 mg/kg	Control	8	21.84 ± 1.60 ^b	1.16 ± 0.07 ^b	5.31 ± 0.32 ^b
		PB	8	21.72 ± 1.30 ^b	1.77 ± 0.25 ^{ab}	8.13 ± 1.01 ^{ab}

Results are expressed as means ± SEM.

^a Significant change in PB-fed mice, based on ANOVA (p<0.05).

^b Significant change in p50^{-/-} mice, based on ANOVA (p<0.05).