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Decreased Longevity and Enhancement of Age-Dependent Lesions in Mice Lacking the Nuclear Receptor Peroxisome Proliferator-Activated Receptor α (PPARα)

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ABSTRACT

The nuclear receptor peroxisome proliferator-activated receptor α (PPARα) is activated by peroxisome proliferators (PP), a large class of structurally diverse xenobiotic chemicals, hypolipidemic drugs, and endogenous lipids. PPARα alters the transcriptional programs of genes whose functions include lipid metabolism, inflammation, cell fate, and stress responses in liver, heart, kidney, and skin. Many of these genes are also under control of PPARα in the absence of exogenous peroxisome proliferator exposure. Mice that lack PPARα (PPARα-null mice) exhibit a number of defects in lipid metabolism and accumulate lipids in the liver. Here, we compared the age-dependent lesions in the liver, kidney, and heart in PPARα-null mice with those observed in wild-type SV129 mice, in the absence of exogenous chemical exposure. Groups of mice were sacrificed, at 6, 12, 18, 21, or 24 months of age, or allowed to age until moribund or found dead. PPARα-null mice had decreased longevity, due to a variety of causes. Statistically significant differences in the occurrence of a number of lesions between strains was observed. Hepatocellular carcinomas and multiple hepatocellular adenomas occurred in PPARα-null mice but not wild type mice. Various nonneoplastic spontaneous aging lesions occurred at higher incidence, shorter latency, or increased severity in PPARα-null mice compared with wild-type mice. In the liver, these included vacuolated hepatocytes and sinusoidal cells and mixed cell inflammation. The kidneys of PPARα-null mice exhibited higher incidences and severities of cortical mineralization. Minimal myocardial mineralization occurred at a higher incidence in PPARα-null mice. Our results imply that PPARα delays the development of some spontaneous lesions associated with aging in the liver, kidney, and heart of SV129 mice.

Keywords. Peroxisome proliferator-activated receptor α; peroxisome proliferator; heart; kidney; liver; aging.

INTRODUCTION

Peroxisome proliferators (PPs) are a large class of structurally heterogeneous industrial and pharmaceutical chemicals, originally identified as inducers of increases in both the size and number of peroxisomes in rat and mouse livers (Lock et al., 1989). The peroxisome proliferator-activated receptors (PPARα, β/δ, γ), a subset of the nuclear receptor superfamily, mediate many if not all of the adaptive consequences of PP exposure (Corton et al., 2000; Hihi et al., 2002). PPs may activate PPARs by binding directly to the receptor, or indirectly through perturbation of lipid metabolism resulting in generation of endogenous PPAR ligands (Duplus and Forest, 2002). In the activated state, PPARs form heterodimers with the 9-*cis* retinoic acid receptor (RXR) leading to interaction with PP response elements in the promoters of target genes (Qi et al., 2000).

The 3 PPAR subtypes, encoded by separate genes have unique tissue distributions, ligand specificities, and ascribed functions. PPARα is expressed in a number of cell types including hepatocytes, cardiomyocytes, kidney proximal tubule epithelial cells, and skin keratinocytes. PPARα plays a key role in mediating the effects of hypolipidemic and xenobiotic PP in these tissues. Exposure to PPs causes a predictable set of responses in rats and mice including increased expression of fatty acid β-oxidation genes, hepatocyte peroxisome proliferation, hepatomegaly, and hepatocyte hyperplasia. Chronic exposure to many PPs causes an increased incidence of liver tumors in male and female mice and rats (Cattley et al., 1998; Klaunig et al., 2003). These responses require a functional PPARα, as PP-exposed PPARα-null mice lack all of the short- and long-term responses including alteration in lipid metabolism, hepatocyte proliferation, and increases in liver tumors (Aoyama et al., 1998; Corton et al., 1998; Fan et al., 2003; Lee et al., 1995; Motojima et al., 1997; Peters et al.,1996, 1997).

Aging is a complex stochastic process determined by genetic and environmental factors (Coffer, 2003; Hamet and Tremblay, 2003; Jaenisch and Bird, 2003; Parsons, 2003; Sharpless and DePinho, 2004). A small number of genes have been identified in mice that, when inactivated, increase longevity possibly through increased ability to respond to acute and chronic environmental stressors (Longo and Finch, 2003). Additional genes reduce the rate of aging, i.e., when the gene is inactivated, there is an earlier occurrence of age-associated lesions and decreased longevity (Warner and Sierra, 2003). In mice, genetic differences in susceptibility to conditions that impair survival, e.g., amyloidosis and megaesophagus (HogenEsch et al., 1996; Ward et al., 2000) influence longevity. As an example of an environmental influence

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on aging, feed restriction increased longevity and decreased the incidences of several types of neoplasm in 3 strains of mice (Sheldon et al., 1996).

There is evidence that PPARα plays multiple roles in the aging process. Older animals exhibit decreases in the expression of PPARα or genes regulated by it in T-lymphocytes (Jones et al., 2002), liver (Perichon and Bourre, 1995, 1996; Youssef and Badr, 1999), and heart (Iemitsu et al., 2002). PPARα activity may decrease indirectly through age-dependent decreases in liver RXR (Chao et al., 2002). PPARα may influence aging through regulation of damage and repair processes after exposure to endogenous or environmental stressors. Aging in experimental animals is associated with increases in oxidative stress, and PPARα may play a role in reducing endogenous oxidative stress in the liver as lipid peroxide levels were increased in 12-month-old PPARα-null mice (Poynter and Daynes, 1998). Wild-type mice preexposed to PPARα agonists exhibit decreased cellular damage, increased tissue repair, and decreased mortality after exposure to a number of physical and chemical stressors in liver (Anderson et al., 2002; Chen et al., 2000; Mehendale, 2000; Shankar et al., 2003; Wheeler et al., 2003). PPARα-null mice were more sensitive to kidney damage after ischemia-reperfusion (Portilla et al., 2000) and prior exposure of wild-type mice to PP reduces ischemia-reperfusion injury (Sivarajah et al., 2002). PPARα agonists decreased damage to skin epithelia from UV damage (Kippenberger et al., 2001). In the heart, PPARα was identified as a downstream effector of p38 kinase-dependent stress-activated signaling, linking extracellular stressors to alterations in energy metabolic gene expression (Barger et al., 2001). PPARα-null mice exhibited age-dependent cardiomyopathy and decreased basal expression of lipid-metabolism genes in the heart (Watanabe et al., 2000). Taken together, these studies indicate that PPARα plays an important role in suppression of endogenous and chemical-induced damage.

Despite the well-known roles of PPARα in mediating the effects of PP exposure, a systematic characterization of the age-dependent lesions in PPARα-null mice has not been carried out. We show here that PPARα-null mice exhibit decreased longevity and enhanced occurrence of a subset of lesions typically found in the aging mouse liver, kidney, and heart.

MATERIALS AND METHODS

Animals

Wild-type and homozygous PPARα-null mice on an SV129 background were used. Mice were originally obtained from the laboratory of Dr. Frank Gonzalez (Lee et al., 1995) and a breeding colony for both the PPARα-null and corresponding wild-type mice was established and maintained at CIIT Centers for Health Research (CIIT) since 1997. The aging study was initiated in 1999. The genetic background of both wild-type and PPARα-null mice was of a mixed SV129/C57BL/6. Mice were provided with NIH-07 rodent chow (Zeigler, Gardners, PA) and deionized, filtered water ad libitum. Lighting was on a 12-hour light-dark cycle. Fifty-six wild-type and 53 PPARα-null male mice (up to 4/cage) were housed in plastic shoebox cages. Groups of mice were sacrificed at 6, 12, 18, 21, and 24 months of age (days 171, 368–369, 541–548, 632–639, and 724–740, respectively), while the others were allowed to age until they were moribund or found dead (premature decedents). All animal studies were conducted under federal guidelines for the use and care of laboratory animals and were approved by the Institutional Animal Care and Use Committee of CIIT.

Pathology Techniques

Mice were sacrificed by exsanguination following intraperitoneal injection of pentobarbital. All mice were subjected to a full necropsy. Livers, kidneys, and hearts were fixed in 10% neutral buffered formalin. Hematoxylin and eosin stained, 5-μ sections were prepared from 3 lobes of liver, transversely through each kidney and longitudinally through the heart and were evaluated histologically. Selected sections were also stained by the periodic acid-Schiff technique, or immunostained using the following methods. Antigen retrieval was carried out, using Decloaker (Biocare Medical, Walnut Creek, CA) for 20 minutes at 95°C for MOMA-2, F4/80 and Factor VIII staining. Primary and secondary antibodies were applied at room temperature for 40 and 30 minutes, respectively, secondary antibodies were visualized by applying diaminobenzidine for 5 minutes, then hematoxylin counterstain was applied for 30 seconds.

For actin staining, primary antibody (monoclonal, mouse anti-human actin, Serotec, Oxford, UK) was diluted 1:50 and the secondary antibody was anti-mouse labeled polymer (Dako, Carpinteria, CA). For F4/80 and MOMA-2 staining, primary antibodies (rat anti-mouse F4/80 antigen and rat anti-mouse macrophages/monocytes, both monoclonal, from Serotec, Oxford, UK) were diluted 1:100 and the secondary antibody was horseradish peroxidase conjugated goat anti-rat IgG (Serotec, Oxford, UK). For Factor VIII staining, the primary antibody (monoclonal, mouse anti-human, Biogenex, San Ramon, CA) was diluted 1:100 and the secondary antibody was anti-mouse labeled polymer (Dako). MOMA-2 staining was not successful. A formalin-fixed sample of liver with hepatocyte vacuolation was also stained for lipid by Oil Red O (ORO).

Analysis of Pathology Findings

The cause of death was assessed from either clinical history (usually, the reason for euthanasia), necropsy findings, or on histological findings in the liver, kidney, or heart (Table 1). The statistical significance of differences in frequency of each cause of death, regardless of age, was assessed using Fisher's test. Animals were placed into groups according to their age at death (regardless of whether they were premature decedents or in planned sacrifices). These groups were: 171–455 days old (including the scheduled sacrifices at 171 and 368–369 days old), 456–678 days old (including the scheduled sacrifices at 541–548 and 632–639 days old) and 679–941 days old (including the scheduled sacrifice at 724–740 days old). The numbers of premature decedents and scheduled sacrifice animals in each age group are indicated in Tables 2, 3 and 4. Nonneoplastic lesions were graded according to severity and extent as minimal, mild, moderate, or marked. Lesions were regarded as enhanced in PPARα-null mice if their incidences or average grades were greater than in wild-type mice in any of the age groups. Age-dependent lesions were defined as lesions that increased in incidence and/or severity with age in this study, or were reported to do so.

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TABLE 1.—Comparison of incidences of causes of death in wild-type and PPARα-null mice.

Strain	WT[a]	Null[b]	WT	Null	WT	Null	WT	Null
Age at death (days)	171–455	171–455	456–678	456–678	679–941	679–941	All	All
Number of premature decedents	4	4	4	11	16	9	24	24
Cause of Death								
Abdominal mass		1						1
Accidental	1				1		2	
Cardiomyopathy			1				1	
Eye lesion				2	1	4	1	6
Harderian gland mass					1		1	
Hemangiosarcoma					2		2	
Hepatocellular carcinoma						1		1
Histiocytic sarcoma			2				2	
Kidney necrosis					1		1	
Lung mass(es)			1		1		2	
Mass on back					1		1	
Megaesophagus				2	3	1	3	3
Paralyzed hindleg				1				1
Paraphimosis	1					1	1	1
Renal adenoma					1		1	
Skin lesion		2		4	2		2	6
Testis mass				1				1
Unknown	2	1		1	1	2	3	4
Urinary bladder distension					1		1	

[a] WT = wild-type.
[b] Null = PPARα-Null.

The statistical significance of the difference in incidence of each finding between wild-type and PPARα-null mice was assessed for each age group, by using Fisher's exact test to test the hypothesis that the responses (grades) were the same in the 2 strains of mice. p-values were not adjusted for multiplicity. Differences were considered statistically significant if the resulting p-values (2-tailed) were <0.05; these are indicated in Tables 2, 3 and 4.

RESULTS

Longevity

Longevity of PPARα-null mice was significantly less than wild-type mice (Wilcoxon test $p < 0.027$; Figure 1). The first 2 premature decedents (2 wild-type mice, sacrificed on days 184 and 194, respectively) were not included in the longevity data, as they were removed from the animal unit without confirmation that their condition required humane sacrifice, and could not then be returned to the unit, for hygiene reasons. These animals are, however, included in the cause of death and histopathological analyses. The causes of death are listed in Table 1. None was statistically significantly enhanced in PPARα-null mice, although skin and eye lesions were slightly more frequent in PPARα-null mice. The cause of death was ascribed with at least reasonable certainty in most cases, except for seven of the 48 premature decedents, where it was unknown.

Necropsy Findings

The only necropsy findings that clearly differed in incidence between wild-type and PPARα-null mice (data not shown) were pallor of, and masses in, the liver (increased in PPARα-null mice up to 455-days-old and over 679-days-old respctively). Preputial gland enlargement was slightly more frequent in wild-type mice at some age intervals. There were significant increases in liver-to-body weights in the PPARα-null mice at 18 months (3.13 ± 0.65 (standard error of the mean), wild-type vs. 6.07 ± 0.22, PPARα-null) and at 24 months (4.35 ± 0.10, wild-type vs. 7.87 ± 1.66, PPARα-null). There were also significant increases in kidney-to-body weights for PPARα-null mice at 24 months (1.2 ± 0.10, wild-type vs. 2.2 ± 0.06, PPARα-null). No significant changes in the organ-to-body weights were observed for adrenal, brain, heart, spleen, and testis.

Histopathological Findings in the Liver (Table 2)

Hepatocellular carcinomas and multiple hepatocellular adenomas occurred only in PPARα-null mice, (multiple adenomas attaining statistical significance in the oldest age group). The incidences and age-occurrence of single hepatocellular adenomas were similar in wild-type and PPARα-null mice. Hepatocellular tumors were diagnosed as carcinomas only if they had trabeculae of hepatocytes 3 or more cells wide. Focal hepatocyte changes (hyperplastic, hypertrophic, or basophilic) each occurred in only 1 mouse (2 of which were wild-type), so there was no indication of an influence of the PPARα gene on these potentially preneoplastic lesions.

Vacuolated cells in or lining the sinusoids (Figure 2) were age-related and had an earlier age of first occurrence in PPARα-null mice and higher incidences and severities in PPARα-null mice than in wild-type mice (statistically significant in the youngest and oldest age groups). The vacuolated cells contained periodic acid-Schiff positive material, which was not glycogen, as it was diastase-resistant. Their cytoplasm immunostained moderately strongly for α-actin, implying that the cells were myofibroblastic-transformed Ito cells. The cells immunostained weakly for Factor VIII (an endothelial cell marker), but were negative for F4/80 (a macrophage marker). Hepatocyte vacuolation (Figures 3, 4) and mixed cell inflammation occurred at higher incidences and/or severities in PPARα-null mice than in wild-type mice (statistically significant in the youngest and oldest age groups), but were not age-related. The vacuoles in hepatocytes were Oil Red O positive. Bile duct hyperplasia and hepatocyte nuclear inclusions occurred at nonsignificantly higher incidences in PPARα-null mice than in wild-type mice. Hepatocyte nuclear inclusions consisted of small portions of cytoplasm invaginated into the nucleus. Bile duct adenomatoid lesions occurred at nonsignificantly higher incidences

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TABLE 2.—Comparison of incidences of selected histopathological findings in the liver in wild-type and PPARα-null mice.

Strain	WT[a]	Null[b]	WT	Null	WT	Null
Age at death (days)	171–455	171–455	456–678	456–678	679–941	679–941
Number of mice in scheduled sacrifices	7 (171)	8 (171)	6 (541)	6 (548)		
(age at death, in days)	7 (369)	8 (368)	5 (632–639)	4 (632–638)	7 (724)	3 (733–740)
Number of premature decedents	4	4	4	11	16	9
Number of animals where liver examined	18	19	15	19	22	12
Finding						
Hepatocellular carcinoma						2
Hepatocellular adenoma, multiple				2		3.037
Hepatocellular adenoma, single			1	2	5	3
Vacuolated sinusoidal cells		*.020*				*<.001*
Minimal		4	4	8	4	4
Mild		2		4	1	7
Moderate				1		
Hepatocyte vacuolation		*<.001*				*<.001*
Minimal	7			4	4	2
Mild	4	5	5	3		6
Moderate		9		1		
Marked		4				
Inflammation, biliary tract		*.011*				
Minimal		5	2	4	5	5
Mild	1	4		4	3	4
Moderate	2			1	1	
Mixed cell inflammation		*.008*				*.015*
Minimal	4	4	4	6	8	2
mild	1	9	2	7	1	3
moderate	2	3		1		3
Bile duct hyperplasia						
Minimal	1	1	2	3	1	3
Mild		1		1		
Hepatocyte nuclear inclusion(s)						
Minimal	7	12	12	14	15	8
Mild	1	3	1	1	3	3
Moderate				1		
Bile duct adenomatoid lesion						
Minimal		3				1
Mild	1	1		2	1	
Moderate	2	2		1		
Necrosis, hepatocyte						
Minimal		2				
Mild			1			
Moderate		1			1	
Hemangiosarcoma					2	
Hemangioma				1		
Angiectasis						
Mild	1			1	2	1
Hyperplasia, sinusoidal cell						
Mild			1	1	4	

[a]WT = wild-type.
[b]Null = PPARα-Null
Numbers in italics are *p*-values for the finding and age group concerned. These are given where <0.05.

in PPARα-null mice than in wild-type mice in the first 2 age categories. This lesion (Figure 5; Harada et al., 1999; Ward et al., 2001) involved many intrahepatic bile ducts in each affected liver section. The duct epithelium was thickened and glandular, with superficial epithelial cells having intensely eosinophilic cytoplasm, while basal epithelial cells contained mucin. Elongated, eosinophilic crystal-like structures were often present in the lumens of affected bile ducts. Three PPARα-null mice up to 455-days-old exhibited hepatocyte necrosis (not statistically significant); 2 older wild-type mice also had this finding.

Hemangiosarcoma, hemangioma, angiectasis, and sinusoidal cell hyperplasia were age-related and were not enhanced in PPARα-null mice. The hyperplastic sinusoidal cells immunostained positive for F4/80 but negative for α-actin, implying that they were macrophages.

Histopathological Findings in the Kidney (Table 3)

Cortical mineralization and cortical cysts (Figure 6) were age-related and exhibited higher incidences and severities in PPARα-null mice than in wild-type mice. Cortical mineralization occurs frequently in old mice and has a complex pathogenesis (Seely, 1999; Ward et al., 2000). Cortical mineralization was statistically significant in the youngest and oldest age groups. The mineralization usually consisted of foci of intracellular or intertubular, laminated or amorphous basophilic material in the deep cortex or in the medulla. The renal cortical cysts may have been related to nephropathy, rather than to congenital renal cystic disease, which can occur in mice (Seely, 1999). Pelvic dilatation was also age-related, although it is usually regarded as a congenital change (Seely, 1999), and had higher incidences and severities in PPARα-null mice in the youngest age group (not statistically significant). Nephropathy is recognized as an age-related change (Seely, 1999; Ward et al., 2000), but the factors that influence its occurrence are far less well understood than they are in rats (Seely, 1999). In the present study, nephropathy was age-related and exhibited nonsignificantly higher incidences and severities in PPARα-null mice than in wild-type mice. Nephropathy usually consisted of foci of tubular basophilia

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TABLE 3.—Comparison of incidences of selected histopathological findings in the kidney in wild-type and PPARα-null mice.

Strain	WT[a]	Null[b]	WT	Null	WT	Null
Age at death (days)	171–455	171–455	456–678	456–678	679–941	679–941
Number of mice in scheduled sacrifices	7 (171)	8 (171)	6 (541)	6 (548)		
(age at death, in days)	7 (369)	8 (368)	5 (632–639)	4 (632–638)	7 (724)	3 (733–740)
Number of premature decedents	4	4	4	11	16	9
Number of animals where kidney examined	18	19	15	19	22	12
Finding						
Mineralization, cortex		*.017*				*.018*
Minimal	3	11	5	6	5	6
Mild				2	1	3
Cyst, cortex						
Minimal				1		2
Mild				1	1	2
Moderate				1		
Pelvic dilatation						
Minimal			1	1	8	3
Mild	3	5	1	3	4	4
Moderate		3	2	2	4	1
Nephropathy						
Minimal	6	8	7	8	9	3
Mild		2		4	3	2
Moderate				1		2
Marked			1			
Inflammation, chronic						
Minimal	5	9	9	7	14	3
Mild			3	7	7	6
Moderate			1	1	1	1
Mineralization, pelvis						
Minimal				1	2	2
Mild			1			

[a]WT = wild-type.
[b]Null = PPARα-Null.
Numbers in italics are *p*-values for the finding and age group concerned. These are given where <0.05.

and dilatation, with eosinophilic tubular contents. Chronic inflammation and pelvic mineralization were also age-related, but occurred at similar incidences and severities in wild-type and PPARα-null mice.

Histopathological Findings in the Heart (Table 4)

Minimal myocardial mineralization (Figure 7) was age-related and occurred at a higher incidence in PPARα-null mice than in wild-type mice (statistically significant in the oldest age group). Cardiomyopathy (Figures 8, 9) was not increased in incidence or severity in PPARα-null mice, but was age-related.

DISCUSSION

The nuclear receptor PPARα regulates a large battery of genes involved in lipid metabolism, inflammation, and cell fate (Corton et al., 2000). There is increasing recognition that PPARα may link control of metabolic pathways involved in energy production with responses to chemically induced stress. As PPARα is required to prevent damage from a number of exogenous stressors, it is reasonable to postulate that PPARα may also suppress the development of age-dependent lesions, some of which result from oxidative stress and associated damage to macromolecules and tissues. This study was intended to investigate whether PPARα-null mice exhibit differences in longevity and the occurrence of age-associated pathological changes in the liver, kidney, and heart, compared to wild-type mice.

In PPARα-null mice, the incidences and severities of several age-related changes were greater and the age of first occurrence earlier in the liver, kidney, and heart. These included hepatocellular tumors and vacuolated sinusoidal cells in the

TABLE 4.—Comparison of incidences of selected histopathological findings in the heart in wild-type and PPARα-null mice.

Strain	WT[a]	Null[b]	WT	Null	WT	Null
Age at death (days)	171–455	171–455	456–678	456–678	679–941	679–941
Number of mice in scheduled sacrifices	7 (171)	8 (171)	6 (541)	6 (548)		
(age at death, in days)	7 (369)	8 (368)	5 (632–639)	4 (632–638)	7 (724)	3 (733–740)
Number of premature decedents	4	4	4	11	16	9
Number of animals where heart examined	18	19	10	17	22	12
Finding						
Myocardial mineralization						.037
Minimal	1	1		4		3
Cardiomyopathy						
Minimal	2	4	2	7	11	3
Mild	1	1	1	2	6	8
Moderate			1	1	2	
Marked				1		

[a]WT = wild-type.
[b]Null = PPARα-Null.
Numbers in italics are *p*-values for the finding and age group concerned. These are given where <0.05

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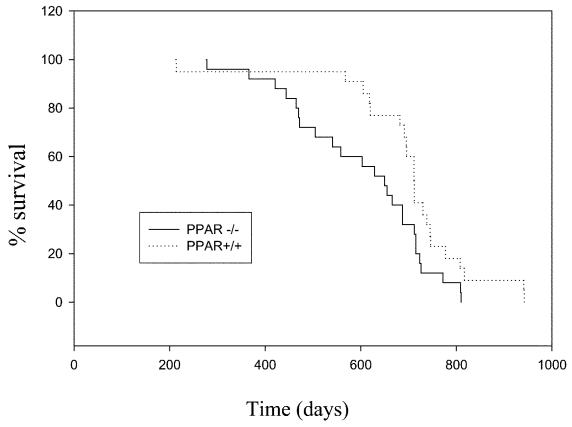

FIGURE 1.—Longevity of male wild-type (+/+) and PPARα-null (−/−) mice.

liver, cortical mineralization, and cortical cysts in the kidney and myocardial mineralization in the heart. Although not clearly age-related in this study, hepatocyte vacuolation is reported to be age-related or frequent in aging mice (Ward et al., 2000) and was also enhanced in PPARα-null mice. The relatively small number of animals available made it difficult to draw robust conclusions concerning enhancement of spontaneous findings in PPARα-null mice. Overall, however, these results imply that PPARα influences the rate at which a number of age-dependent lesions occur, presumably by affecting molecular processes that determine age-dependent tissue damage. The incidence of the enhanced findings in PPARα-null mice was seldom near 100%, and these findings were not unique to PPARα-null mice. This is consistent with PPARα as one of many factors which influences the development of aging lesions.

There is evidence for several mechanisms by which PPARα may protect tissues from acute or chronic stress. The first involves protection of proteins from damage associated with oxidative stress. Support for this comes from recent transcript profile studies in which a number of classes of proteins involved in maintaining the integrity of the proteome were found to be regulated by PPARα (Anderson et al., submitted). These include chaperone and chaperonin proteins that bind to unfolded proteins, preventing aggregation and associated cytotoxicity and proteasome family members involved in degrading damaged or excess proteins (Anderson et al., submitted).

A second mechanism involves the suppression of age-dependent increases in inflammatory cytokines and associated inflammation that contribute to a number of pathological states. PPARα negatively regulates the activity of transcription factors that control the expression of inflammatory cytokines through direct interaction and seqestration of the NF-κB subunit, p50 as well as c-Jun (Corton et al., 2000). PPARα also indirectly controls the levels of inflammatory lipid mediators such as LTB_4 and its precursor arachidonic acid catabolized through the β-oxidation pathway (Devchand et al., 1996). Decreases in PPARα expression in T-lymphocytes were associated with increases in the activity of NF-κB and expression of NF-κB-regulated inflammatory cytokines (Poynter and Daynes, 1998). Increases in inflammatory indicators in older mice could be reversed by treatment of wild-type but not PPARα-null mice with PPARα agonists (Poynter and Daynes, 1998). Deregulated suppression of inflammatory responses in PPARα-null mice may have contributed to the increased incidence of mixed cell inflammation in our study.

PPARα may also protect the liver from damage through regulation of hepatocyte proliferation, as PPARα-null mice exhibited defects in repair of liver damage through decreased regenerative hepatocyte proliferation compared to wild-type mice (Shankar et al., 2003). Consistent with this, PPARα-null mice exhibit a delay in liver regeneration after a two-thirds partial hepatectomy (Anderson et al., 2002; Wheeler et al., 2003) although not consistently in all studies (Rao et al., 2002).

Hepatocyte lipid vacuolation and vacuolated hepatic sinusoidal cells (apparently of Ito cell origin) were the most clearly enhanced nonneoplastic lesions in PPARα-null mice in this study. Vacuolated sinusoidal histiocytic cells have been induced in the mouse liver as part of systemic phospholipidosis (Stebbins et al., 2002) and the precise identity of the material in the sinusoidal cells in the present study is unclear. Lipid catabolism is deficient in PPARα-null mice (Corton et al., 2000; Poynter and Daynes, 1998), resulting in centrilobular lipid accumulation observed in 30-week-old mice (Ward et al., 1997) . Hepatocyte vacuolation and vacuolated sinusoidal cells occurred in wild-type mice, although at a lower incidence and severity than in PPARα-null mice. Ward et al. (2000) also noted that hepatocellular vacuolation occurs at an incidence of 17.5% in aging, wild-type 129S4/SvJae male mice. The present results therefore demonstrate that the effects of lack of the PPARα gene on lipid metabolism in the liver must be assessed in relation to liver function in the background strain.

As exposure to strong PPARα activators has been associated with increased incidences of liver neoplasia in mice and rats (Klaunig et al., 2003), it was surprising that PPARα-null mice exhibited hepatocellular carcinomas and multiple adenomas, which were not seen in wild-type mice (Table 2). This finding, in particular, requires confirmation by the use of more animals. However, the increased incidence is consistent with the fact that exposure to WY-14,643 can suppress the growth of certain types of preneoplastic foci in the rat liver (Cattley et al., 1989). Thus, at least in the liver, there may be a "U"-shaped relationship between tumor incidences and PPARα activation, i.e., increases in tumor frequency at either no PPARα activation or high activation under conditions of chronic exposure to strong PP.

PPARα-null mice exhibit abnormalities in myocardial fatty acid transport and metabolism and age-dependent myocardial fibrosis at 16 and 32 weeks of age (i.e., 112 and 224 days), which were not observed in wild-type mice (Watanabe et al., 2000). In the present study, however, cardiomyopathy was also present in wild-type mice and was not distinctly enhanced in PPARα-null mice. We suggest that either differences in genetic background or in animal husbandry between our study and that of Watanabe et al. (2000) may alter the age-dependent changes in the hearts of PPARα-null mice. We observed minimal myocardial mineralization in several PPARα-null mice and in only 1 wild-type mouse. Although genetics, age, and feeding large amounts of fat could

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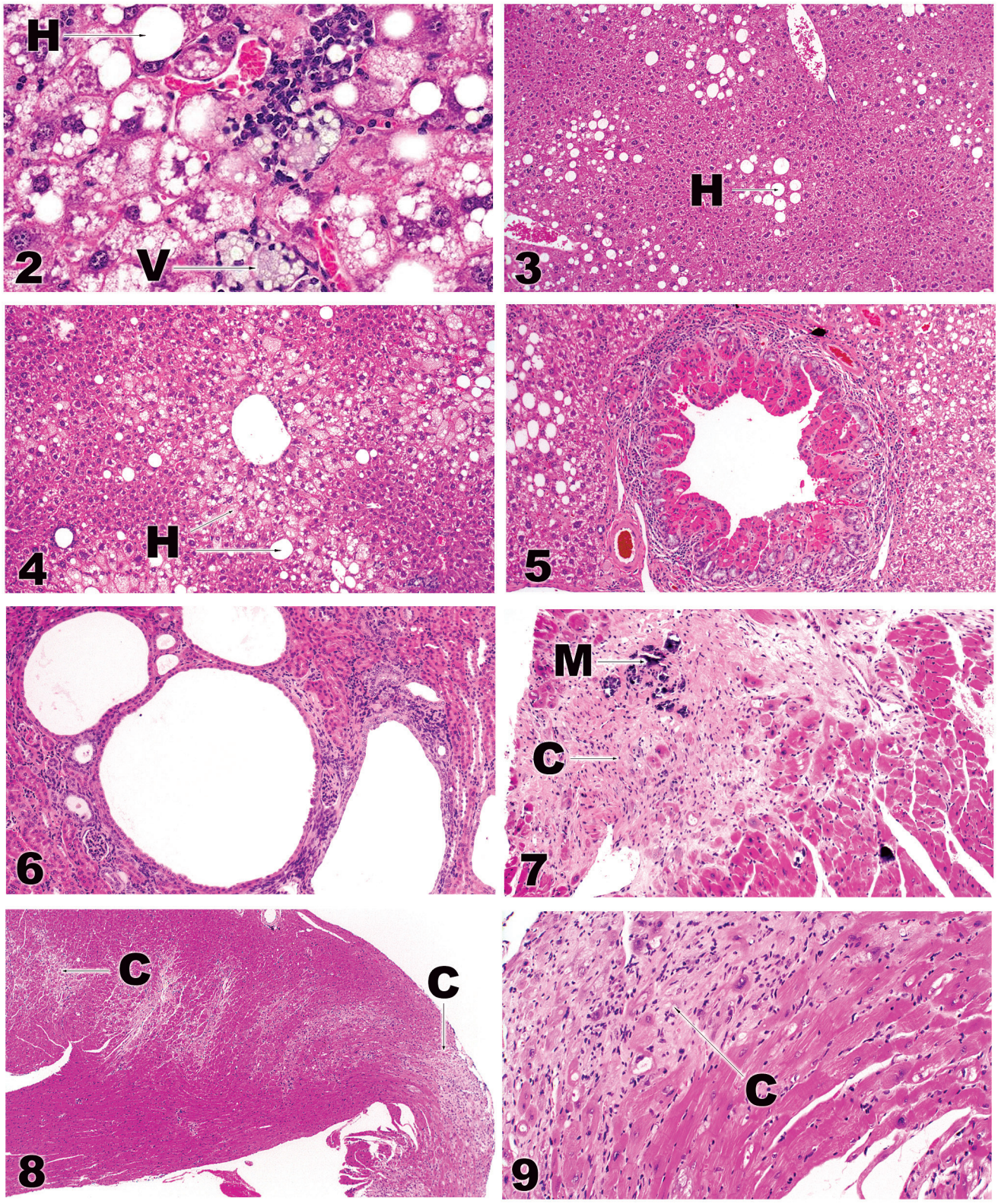

Figures 2–9

FIGURE 2.—Liver. Vacuolated sinusoidal cells (V); also hepatocyte vacuolation (H). PPARα-null mouse, day 632. H & E, ×800. 3.—Liver. Mild hepatocyte vacuolation (H). Wild-type mouse, day 541. H & E, ×200. 4.—Liver. Marked hepatocyte vacuolation (H). PPARα-null mouse, day 368. H & E, ×200. 5.—Liver. Moderate bile duct adenomatoid lesion. PPARα-null mouse, day 171. H & E, ×200. 6.—Kidney. Moderate cortical cysts. PPARα-null mouse, day 603. H & E, ×200. 7.—Heart. Minimal myocardial mineralization (M) and mild cardiomyopathy (C). PPARα-null mouse, day 715. H & E, ×200. 8.—Heart. Moderate cardiomyopathy (C). Wild-type mouse, day 941. H & E, ×80. 9.—Higher magnification of Figure 8. Moderate cardiomyopathy (C). ×400.

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influence the degree of cardiac mineralization (Eaton et al., 1978), the pathogenesis of this change in the present study is unclear. Mineralization has been associated with cardiac myofiber necrosis and calcification in the kidney and lung (Eaton et al., 1978), whereas in the present study, the mineralization was often associated with cardiomyopathy (although not with necrosis) and there was no clear relationship with renal mineralization.

Various skin and orbital lesions can necessitate euthanasia in aging mice (Sundberg et al., 1996; Botts et al., 1999; Peckham and Heider, 1999; Ward et al., 2000); such lesions probably accounted for the skin, eye, and Harderian gland lesions in this study, although they were not investigated histologically. Ward et al. (2000), reported that esophageal impaction/megaesophagus caused the death of 3/40 male 129S4/SvJae mice, which is similar to the incidence of 6/108 mice in this study.

In summary, PPARα reduces the severity and frequency of a subset of age-dependent lesions in the mouse. These findings in the liver, kidney, and heart, in which PPARα is normally expressed and mediates regulation of gene expression, indicate that PPARα in addition to regulation of lipid metabolizing genes, regulates genes involved in maintaining the integrity of these tissues. Future work will be focused on linking the involvement of proteome maintenance and inflammation control genes in the alteration of stress responses and aging.

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