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Phenotype of peroxisome proliferator-activated receptor-α (PPARα) deficient mice on mixed background fed high fat diet

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Considerable controversy exists in determining the role of peroxisome proliferator-activated receptor-α (PPARα) on obesity. Previous reports demonstrated that PPARα is a critical modulator of lipid homeostasis, but the overt, obese phenotypic characterization in the strain of PPAR deficient (PPARα-/-) mice is influenced by other factors, including diet and genetics. Therefore, it is necessary to establish the phenotypic characterization of PPARα-/- mice prior to the obesity-related study. In this study, we observed phenotype of PPARα-/- mice on mixed genetic background (C57BL/6N×129/Sv) fed a high fat diet for 16 weeks. PPARα-/- mice, regardless of sex, raised body growth rate significantly comparing with wild type and showed male-specific fatty change in the liver. They were shown to lack hepatic induction of PPARα target genes encoding enzymes for fatty acid β-oxidation.

Key words: Phenotype, PPARα-/- mice, C57BL/6N×129/Sv, high fat diet

Introduction

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily which form heterodimers with the retinoid X receptor [4,8]. They play an important role in the regulation of genes involved in lipid utilization and storage, lipoprotein metabolism, adipocyte differentiation, and insulin action. There are three subtypes of PPARs, α, δ and γ, that have distinct tissue distribution patterns. PPARα is mainly present in the liver, heart, and kidney. PPARδ is ubiquitously expressed, whereas PPARγ is predominantly expressed in adipose tissue [2]. Several endogenous ligands and drugs have been identified that bind to PPARs and activate gene transcription; Fatty acids, fibrates, Wy-14643, and leukotriene B4 preferentially bind to PPARα [8]; PPARγ is activated by polyunsaturated fatty acids, prostaglandin J2, and thiazolidinedione group of drugs [7].

Generation of PPARα deficient (PPARα-/-) mice established that the receptor is a prerequisite for hepatic peroxisome proliferation and coordinatie induction of acyl-coenzyme A oxidase (ACO), bifunctional enzyme (HD), thiolase (THL), cytochrome P450 4A, and liver fatty acid-binding protein genes by clofibrate [5,6]. Although PPARα is a critical modulator of lipid homeostasis, its role in obesity remains to be fully substantiated. Actually, PPARα-/- mice were shown different sexual dimorphic obese phenotype depending on their genetic background and diet [1,3]. Therefore, it is necessary to document the phenotypic characterization of PPARα-/- mice under the various environments, prior to study the putative influence of PPARα on obesity. In this study, we observed phenotype of PPARα-/- mice on mixed background (C57BL/6N×129/Sv) fed high fat diet for 16 weeks comparing with wild-type (PPARα+/+) mice; body growth rate, liver and white adipose tissue (WAT) change, plasma lipid level, and expression of PPARα target genes encoding peroxisomal β-oxidation, ACO, HD and THL.

Materials and Methods

Experiemetal animals and genotyping

Specific pathogen free C57BL/6N×129/Sv homozygous PPARα+/+ and PPARα-/- mice were initially introduced from National Institutes of Health (Bethesda, MD, USA) and bred at Korea Research Institute of Bioscience and Biotechnology. Mice were weaned at 28 days, fed *ad*

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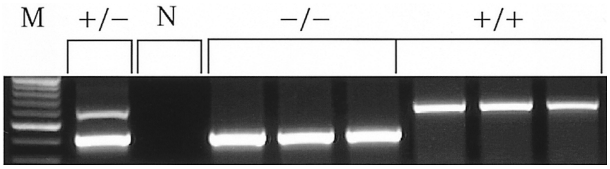

Fig. 1. Genotyping by polymerase chain reaction in PPARα+/+ and PPARα-/- mice. Genomic DNA produced a band of 650 bp in wild type, a mutant band of 400 bp in PPARα-/- mice and both wild and mutant band in control heterozygote mice;. M = 100 bp marker, +/- = heterozygote (control), N = negative control, -/- = PPARα-/- mice, +/+ = PPARα+/+ mice.

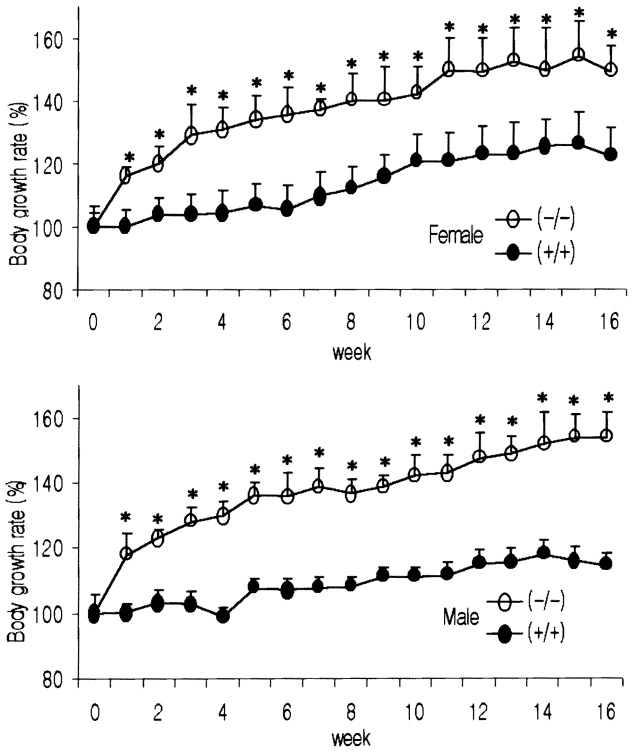

Fig. 2. The comparison of body growth rate between wild and PPARα-/- mice. Body growth rate was expressed as the value relative to body weight (B.W.) of each group at day 0 ((B.W. at each week/ B.W. at week 0) X100). A; female, B; male.
*P<0.0005. Significantly different from wild type.

libitum a standard mouse chow diet, and housed in autoclaved polycarbonate cages with wire lids. All feed, water and beddings were sterilized by autoclaving. The animal room was kept between 20 to 22°C under a 12-h light-dark cycle.

Their genotypes were confirmed by amplification of genomic DNA from tail samples; genomic DNA were prepared from tails of mice by incubation with proteinase K (100 g/ml) in digestion buffer (0.45% Tween 20, 0.45% NP-40, 50 mM KCl, 10 mM TrisCl, 2.5 mM $MgCl_2$) overnight at 55°C followed by extraction with phenol/chloroform and ethanol precipitation. The nucleotide sequences of PCR primers used for genotyping were the PPAR forward primer, 5'-GAGAAGTTGCAGGAGGGGATTGTG-3', the reverse primer, 5'-CCCATTTCGGTAGCAGGTAGTCTT-3', and the NEO primer 5'-GCAATCCATCTTGTTCAATGGC-3'. The thermal cycles were as followings; 1 cycle at 94°C for 5 min, followed by 35 cycles each at 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min, and 1 additional cycle at 72°C for 5 min. The PCR products were electrophoresed on a 1.5 % agarose gel (Fig. 1).

Phenotypic characterization

For obese phenotypic observation, six to eight week-old male or female PPARα+/+ and PPARα-/- mice were fed on high fat diet containing 15% fat, 1.25% cholesterol, 0.5% Na-Cholate (Oriental Yeast CO, Ltd, Japan) for 16 weeks. Their body weights were measured weekly and blood was collected at week 1, 4, 8, 16 post high fat diet. After 16 weeks, mice were sacrificed by cervical dislocation: the liver and the visceral white adipose tissue (WAT) were excised, weighed, snap frozen in liquid nitrogen, stored at −70°C until use and fixed in 10% phosphate-buffered formalin. The livers processed in a routine manner for paraffin-section, cut to 3 μm, and stained with hematoxylin and eosin for microscopic examination. Blood was collected from the retro-orbital

Table 1. Body, liver, and white adipose tissue weight (wt) in PPARα+/+ and PPARα-/- mice. n, number of mice examined; B.W., body weight. Weights of liver and visceral white adipose tissue (WAT) were expressed as grams relative to body weight (g/g B.W.)

Sex	Genotype	n	B.W. (g)		Liver Wt (g/g B.W.)	Visceral WAT (g/g B.W.)
			Week 0	Week 16	Week 16	Week 16
Female	(+/+)	7	21.45 ± 0.88	26.23 ± 1.86	0.067 ± 0.007	0.039 ± 0.008
	(-/-)	11	16.40 ± 1.02	24.40 ± 1.40	0.081 ± 0.005	0.037 ± 0.009
	% of (+/+)	-	-	-	120.9%[a]	94.90%
Male	(+/+)	9	25.28 ± 0.23	28.85 ± 1.00	0.056 ± 0.004	0.030 ± 0.010
	(-/-)	8	19.00 ± 1.01	29.10 ± 1.48	0.107 ± 0.015	0.030 ± 0.000
	% of (+/+)	-	-	-	191.1%[b]	100%

Results were expressed as mean ± SD for all values. [a]P<0.005, [b]P<0.0005.

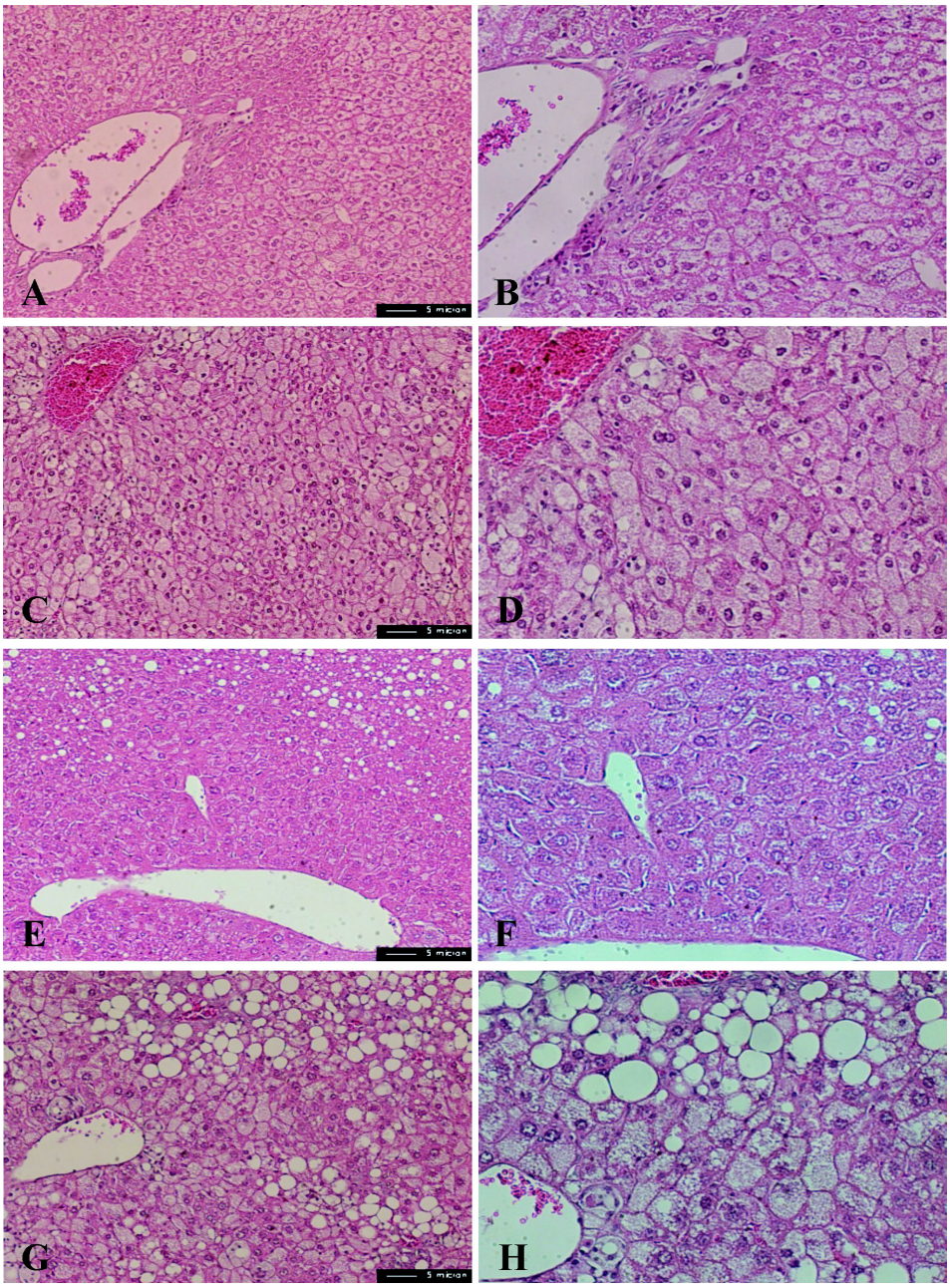

Fig. 3. Histological analyses of livers from male or female PPARα+/+ and PPARα-/- mice at week 16. A-B, female PPARα+/+ mice; C-D, female PPARα-/- mice; E-F, male PPARα+/+ mice; G-H, male PPARα-/- mice; X100 (A, C, E, and G), X400 (B, D, F, and H); H&E stain.

venous plexus with heparinized capillary tube, and plasma was obtained by centrifugation of the whole blood at 12,000 rpm at 4°C for 10 min and stored at −70°C before analysis. The level of plasma total cholesterol, TG, HDL and LDL were measured using automatic blood chemical analyzer (7020, Hitachi, Japan).

Total RNAs were isolated from liver of all mice using TRIZOL (Gibco BRL, Rockville, MD, USA) according to the manufacturer's recommended procedure. cDNA was synthesized by RT from 1 μg of total RNA using moloney murine leukemia virus RT (Invitrogen, Carlsbad, CA, USA). 2 μl of synthesized total cDNA were used as templates for PCR, and PCR was carried out with Taq polymerase in DNA thermal cycler (Bio Rad, Hercules, California, USA). The sequences of the sense and antisense primers were: 5'-ACTATATTTGGCCAATTTTG TG-3' and 3'-TGTGGCAGTGGTTTCCAAG-5' for ACO, 5'-CAAAAAGATCGGAAAGATT-3' and 3'-CTGATACC

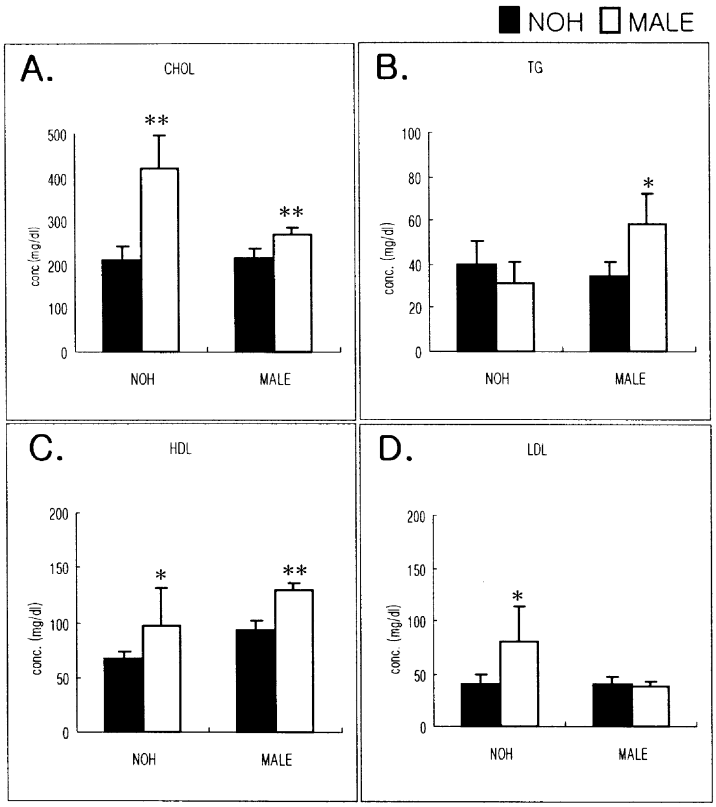

Fig. 4. Plasma lipids level in male and female PPARα+/+ and PPARα-/- mice at week 16. Results are expressed as means ± SD. A, CHOL, total cholesterol; B, TG, triglycerides; C, HDL, high density lipoprotein CHOL; D, LDL, low density lipoprotein CHOL.

AGCCTTTACCT-5' for HD, 5'-AAATGGGTCTTACGAC ATT-3' and 3'-CACTCACCTGACTGGAGTT-5' for THL, and 5'-TGGAATCCTGTGGCATCCATGAAAC-3' and 3'-TAAAACGCAGCTCAGTAACAGTCCG-5' for β-actin. PCRs yielded products of 195, 355, 425 and 349 bp for ACO, HD, THL, and β-actin, respectively.

The amplification products were electorphoretically separated on a 1.5% agarose gel containing ethidium bromide. UV-stimulated fluorescence was captured using a digital video camera and quantitated with the Bio 1D software (Vilber Lourmat, Marine, Cedex, France). Linearity of the PCR was tested by amplifying each cDNA at various numbers of cycles and was found to be between 25 and 35 cycles. All experimental values were normalized to the β-actin.

Results

Individual weight during the 16 weeks revealed significant difference of body weight gain between PPARα+/+ and PPARα-/- mice. It was significantly higher 39.3% in male and 26.6% in female PPARα-/- mice than that of male or female PPARα+/+ mice, respectively (P<0.0005), but had no sexual difference (Fig. 2).

Liver and WAT weights were expressed as values relative to body weight. They were compared between genotypes and expressed as a percentage of the wild-type tissue mass (Table 1). Liver weight and morphological analyses showed a male-specific change in PPARα-/- mice. The liver weight from female PPARα-/- mice represented 120 % of wild-type liver weights, while male mice showed no less than 191% (P<0.0005). On histopathology, the liver of male PPARα-/- mice had severe fatty change with various fat droplets compared to wild type in the cytoplasms (Fig. 3E-H). There were many macrovesicles in the cytoplasm of hepatocytes, and some vacuoles coalesced, creating cleared spaces that displace the nucleus to the periphery of the cell. However, the degree of fatty change in female mice was much mild, and there were no significant difference between genotypes (Fig. 3A-D). On the other hand, WAT weights did not significantly differ between both genotypes and sexes.

Plasma concentrations of cholesterol and HDL were significantly higher in PPARα-/- mice than in wild type. Female PPARα-/- mice exhibited a markedly high level of cholesterol, 197 % of female wild-type values (P<0.0005),

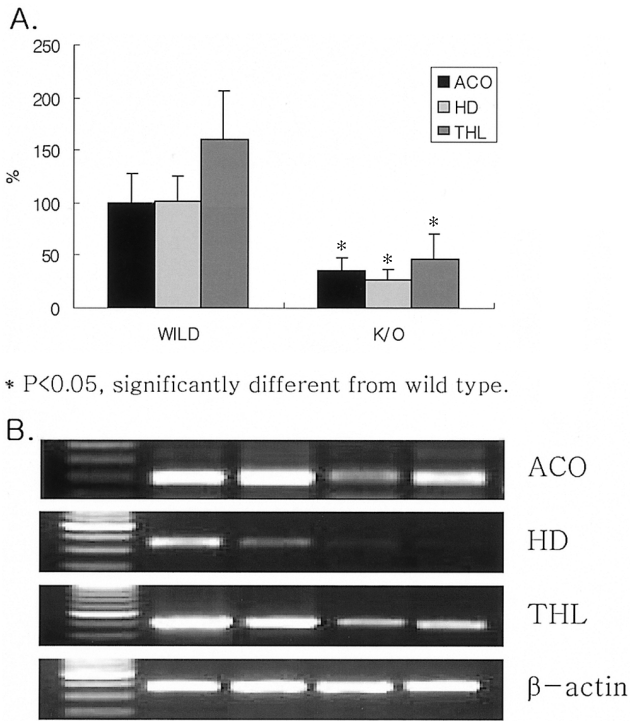

Fig. 5. RT-PCR analyses of hepatic mRNAs encoding enzymes for fatty acid β-oxidation. A. Vertical bars represent the level of mRNA of different genes after normalization to the levels of β-actin mRNA in the same samples. Each values normalized to the β-actin (a) were expressed again as percentage (b) of ACO mRNA level of wild type (b=(a/value of wild ACO)X100). Acyl Co A oxidase (ACO), enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase (HD), 3-ketoacyl-CoA thiolase (THL). B. PCR bands of each gene. Lane 1, 100 bp marker; lane 2, female PPARα+/+ mice; lane 3, male PPARα+/+ mice; lane 4, female PPARα-/- mice; lane 5, male PPARα-/- mice.

but HDL level was no apparent difference in the magnitude of these effects between sexes. Plasma levels of TG were similar in female, while higher in male PPARα-/- mice ($P<0.05$). Plasma levels of LDL were higher in female ($P<0.05$), while similar in male PPARα-/- mice (Fig. 4).

Discussion

PPARα regulates genes encoding the various enzymes in the peroxisomal-oxidation pathway. This enzymatic pathway is responsible for the metabolism of long-chain fatty acids and involves sequentially the enzymes ACO, HD, and THL. We determined the expression level of three genes in liver of male or female PPARα+/+ and PPARα-/- mice by RT-PCR. The values were expressed as percentage of ACO mRNA value of wild type after normalization to the levels of β-actin mRNA in the same sample. The degree of expressions of all three genes were stronger in PPARα+/+ mice than that of PPARα-/- mice ($P<0.05$) (Fig. 5), and there was no significant difference between sexes (data not shown).

The original phenotypic assessment of PPARα-/- mice on a mixed genetic background (C57BL/6N×129/Sv) provided strong in vivo evidence that PPARα mediates the pleiotropic response to peroxisome proliferators, including hepatomegaly, peroxisome proliferation, and induction of genes encoding peroxisomal and microsomal lipid-metabolizing enzymes [6]. Although constitutive expression of peroxisomal and microsomal lipid-metabolizing enzymes was not influenced by targeted disruption of the PPARα gene, hepatic accumulation of lipids was described in PPARα-/- mice, suggesting that constitutive lipid homeostasis is altered in the absence of a functional PPARα [5]. This response is similar between male and female PPARα-/- mice on either a pure 129/Sv or C57BL/6N genetic background [1]. However, the obese phenotypic characterization of PPARα-/- mice was influenced by other factors, including diet and genetics. PPARα-/- mice with unclear genetic background had a late onset, sexually dimorphic, and obese phenotype [5]. PPARα-/- mice with congenic lines showed no significant change of body and adipose weights and different levels of serum lipids depending on their genetic background [7]. And the PPARα-/- mice used in this study (mice on mixed genetic background (C57BL/6N×129/Sv) fed high fat diet) showed significant body weight gain and sexual dimorphic liver change.

These findings point that it is critical to indicate the source of diet and the strain of PPARα-/- mice used for analysis, especially in case of obesity related-studies. Our study could be a good reference which establish the obese-phenotypic characterization of PPARα-/- mice under the mixed genetic background (C57BL/6N×129/Sv) fed high fat diet for 16 weeks.

Acknowledgments

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