Constitutive Regulation of Cardiac Fatty Acid Metabolism through Peroxisome Proliferator-activated Receptor α Associated with Age-dependent Cardiac Toxicity*

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The peroxisome proliferator-activated receptor α (PPARα) is a member of the nuclear receptor superfamily and mediates the biological effects of peroxisome proliferators. To determine the physiological role of PPARα in cardiac fatty acid metabolism, we examined the regulation of expression of cardiac fatty acid-metabolizing proteins using PPARα-null mice. The capacity for constitutive myocardial β-oxidation of the medium and long chain fatty acids, octanoic acid and palmitic acid, was markedly reduced in the PPARα-null mice as compared with the wild-type mice, indicating that mitochondrial fatty acid catabolism is impaired in the absence of PPARα. In contrast, constitutive β-oxidation of the very long chain fatty acid, lignoceric acid, did not differ between the mice, suggesting that the constitutive expression of enzymes involved in peroxisomal β-oxidation is independent of PPARα. Indeed, PPARα-null mice had normal levels of the peroxisomal β-oxidation enzymes except the D-type bifunctional protein. At least seven mitochondrial fatty acid-metabolizing enzymes were expressed at much lower levels in the PPARα-null mice, whereas other fatty acid-metabolizing enzymes were present at similar or slightly lower levels in the PPARα-null, as compared with wild-type mice. Additionally, lower constitutive mRNA expression levels of fatty acid transporters were found in the PPARα-null mice, suggesting a role for PPARα in fatty acid transport and catabolism. Indeed, in fatty acid metabolism experiments in vivo, myocardial uptake of iodophenyl 9-methylpentadecanoic acid and its conversion to 3-methylnonanoic acid were reduced in the PPARα-null mice. Interestingly, a decreased ATP concentration after exposure to stress, abnormal cristae of the mitochondria, abnormal caveolae, and fibrosis were observed only in the myocardium of the PPARα-null mice. These cardiac abnormalities appeared to proceed in an age-dependent manner. Taken together, the results presented here indicate that PPARα controls constitutive fatty acid oxidation, thus establishing a role for the receptor in cardiac fatty acid homeostasis. Furthermore, altered expression of fatty acid-metabolizing proteins seems to lead to myocardial damage and fibrosis, as inflammation and abnormal cell growth control can cause these conditions.

Long chain fatty acids are one of the major cardiac energy substrates, so understanding long chain fatty acid metabolism may help in elucidating the mechanisms of various heart diseases (1–3). Changes in peroxisomal and microsomal gene expression induced by peroxisome proliferators are mediated by the peroxisome proliferator-activated receptor α (PPARα), a member of the nuclear receptor superfamily (4–8). Three distinct PPARs have been found, PPARα, PPARβ (also called PPARγ), and PPARγ. The tissue distribution of each receptor is different, implying that each has unique functions (8). In rodents, PPARα is abundant in the liver, kidney, and heart, all of which have high rates of fatty acid metabolism (8). Little is known about the regulation of fatty acid metabolism in the heart, although such information may help to elucidate the regulatory systems and the physiological roles of PPARα in heart.

Interest in the clinical use of iodine-123-labeled fatty acids is currently primarily focused on the use of iodine-123 15-(p-iodophenyl) pentadecanoic acid and modified fatty acid analogues such as 15-(p-iodophenyl)-3-R,S-methylpentadecanoic acid, which show delayed myocardial clearance, thus permitting single photon emission tomographic imaging (9, 10). Recently, 15-p-iodine-123 iodophenyl 9-methylpentadecanoic acid

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The abbreviations used are: PPAR, peroxisome proliferator-activated receptor; 9MPA, 9-methylpentadecanoic acid; 3MNA, 3-methylnonanoic acid; PIPA, p-iodophenyl acetic acid; H-FABP, heart-type fatty acid-binding protein; VLCAD, very long chain acyl-CoA dehydrogenase; LCAD, long chain acyl-CoA dehydrogenase; LACS, long chain acyl-CoA synthetase; TPC and TPS, mitochondrial trifunctional protein α and β subunit; FATP, fatty acid transport protein; FAT, fatty acid translocase; MCAD, medium chain acyl-CoA dehydrogenase; SCAD, short chain acyl-CoA dehydrogenase; SCHAD, short chain 3-hydroxyacyl-CoA dehydrogenase; CPT II, carnitine palmitoyl-CoA transferase.
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(9MPA), a new single photon agent, has been developed (9–11). 9MPA is a modified long chain (15 carbons, C-15) fatty acid, which differs from idophenyl pentadecanoic acid by a methyl branch at carbon 9. After venous injection, 9MPA is transferred to a myocardial triglyceride pool or undergoes β-oxidation three times in mitochondria, and a metabolite of 9-p-iodophenyl-3-methylnoanilic acid (3MNA) appears. Continuously, 3MNA undergoes α- and β-oxidation, to yield the metabolite of p-iodophenylacetic acid (PIPA). 9MPA is therefore well served to enter into the abilities of fatty acid uptake and oxidation in vivo.

In the present study, we analyzed cardiac fatty acid metabolism both in vitro and vivo, using PPARα-null mice, finding a key role for PPARα in fatty acid metabolism and homeostasis. Additionally, we found that altered fatty acid metabolism, as well as inflammation and abnormal cell growth control, can cause cardiac tissue damage.

METHODICAL PROCEDURES

Materials—[1-14C]Octanoic acid (2 GBq (54 mCi)/mmol), [1-14C]palmitic acid (2 GBq/mmol), and [1-14C]lignoceric acid (1.7 GBq/mmol) were purchased from American Radiolabeled Chemicals (St. Louis, MO). 125I-Labeled 9MPA (7.4 GBq/mg), 125I-labeled 3MNA (7.4 GBq/mg), and 125I-labeled PIPA (7.4 GBq/mg) were donated by Daiichi Radioisotope Laboratories Ltd. (Tokyo, Japan).

Animals—PPARα-null mice in an Sv/129 genetic background were produced as described (12). Wild-type Sv/129 were used as controls in all experiments. The animals were housed five per cage and allowed free access to tap water and standard laboratory mouse chow (Oriental Japan Inc. Tokyo, Japan). Mice were housed in a temperature-controlled room (22 ± 2 °C) under a 12h light/dark (7 p.m. to 7 a.m.) cycle. After determination of the heart rate and blood pressure by the tail cuff method (Softron Co., Tokyo, Japan) at the age of 16 or 32 weeks, the mice were killed.

Mice at the age of 16 weeks were used in the experiments involving stress. Mice were fasted for 48 h and then fed for 24 h. Mice again were fasted for 48 h and then used to analyze ATP, calcium, and magnesium concentrations in myocardium. Following starvation plus high temperature stress, the mouse was placed into a 50-ml plastic tube with many small holes and exposed at 33 °C for 1 h in an air incubator. It was then immediately used for the analysis. All mice survived the stress. The outcome of PPARα-null mice died of hyperthermia when exposed at 42 °C for 20–50 min.

Fatty Acid β-Oxidation Activity—Fatty acid β-oxidation activity was measured by the method of Shindo et al. (13). Briefly, unfrozen myocardial homogenates were sonicated in four volumes of 0.25 M sucrose containing 1 mM EDTA in a Potter-Elvehjem homogenizer using a tightly fitting Teflon pestle. Approximately 500 µg of homogenate was incubated with the enzyme suspension in 0.2 ml of 150 mM potassium chloride, 10 mM HEPES, pH 7.2, 0.1 mM EDTA, 1 mM potassium phosphate buffer, pH 7.2, 5 mM Tris malate, 10 mM magnesium chloride, 1 mM carnitine, 0.15% bovine serum albumin, 5 mM ATP, and 50 µM each fatty acid (5.0 × 103 cpn of radioactive substrate). The reaction was run for 30 min at 25 °C and stopped by the addition of 0.2 ml of 0.6 N perchloric acid. The mixture was centrifuged at 2,000 × g for 10 min, and the unreacted fatty acid in the supernatant was removed with three extractions of 2 ml of n-hexane. Radioactive degradation products in the water phase were counted, and fatty acid β-oxidation activity was expressed as nanomoles/min/mg of protein.

Analysis of the Level of Fatty Acid-metabolizing Proteins—Myocardial extracts were subjected to 10% or 15% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The membranes were incubated with the primary antibody followed by alkaline phosphatase-conjugated goat anti-rabbit IgG. The origin of primary rabbit polyclonal antibodies was described elsewhere (12). Rabbit polyclonal antibody against the heart-type fatty acid-binding protein (H-FABP) was prepared as described previously (14).

mRNA Analysis—mRNA analysis was performed by Northern blotting. Total myocardial RNA was extracted, electrophoresed on 1.1 mM formaldehyde-containing 1% agarose gels, and transferred to nylon membranes (15). The membranes were incubated with 32P-labeled cDNA probes and analyzed on a Fuji system analyzer (Fuji Photo Film Co., Tokyo, Japan). The cDNA probes used were for very long chain acyl-CoA dehydrogenase (VLCAD) (15), long chain acyl-CoA dehydrogenase (LCAD) (16), long chain acyl-CoA synthetase (LACS) (17), mitochrondial trifunctional protein β subunit (TPPβ) (18), fatty acid transport protein (FATP) (19), and fatty acid translocase (FAT) (20).

RESULTS

Analysis of the Level of Fatty Acid-metabolizing Proteins and H-FABP—To identify the level of specific fatty acid-metabolizing enzymes and H-FABP that might be influenced by PPARα, antibodies were used to measure protein levels by immunoblotting (Fig. 1, and Table 1). Constitutive expression levels of several enzymes (VLCA, medium chain acyl-CoA dehydrogenase (MCAD), short chain acyl-CoA dehydrogenase (SCAD), mitochondrial trifunctional protein a subunit (TPα), short chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD), LACS, carnitine palmitoyl-CoA transferase (CPT II), and peroxisomal H-type bifunctional proteins were much lower; 19–77%, in PPARα-null mice than wild-type mice. Some other mitochondrial, microsomal, and cytosolic β-oxidation involving proteins examined and H-FABP were expressed at similar levels in PPARα-null mice and wild-type mice. When comparing the protein contents with those in liver, outstanding reductions in heart were observed for five mitochondrial β-oxidation enzymes (MCAD, SCAD, TPα, SCHAD, and CPT II) (Table 1), indicating constitutive organ-specific expression in a limited
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Expression of mRNAs—To determine whether the drop in the expression level of fatty acid-metabolizing enzymes is due to altered gene expression, myocardial mRNA levels were analyzed by Northern blotting (Fig. 2). Constitutive levels of mRNA for VLCAD and LACS in the PPARα-null mice were 32.7 ± 2.5% \((p = 0.0002)\) and 21.6 ± 1.0% \((p = 0.0004)\) of those in the wild-type mice, respectively. Those for LCAD and TPα were 101.6 ± 3.2% \((p = 0.2511)\) and 116.5 ± 6.9% \((p = 0.055)\), respectively, similar between the two strains. These results are consistent with the protein measurements. It is noteworthy that myocardial levels of mRNA for FATP and FAT in the PPARα-null mice were 51.6 ± 4.4% \((p = 0.0034)\) and 43.8 ± 5.6% \((p = 0.0028)\) of those in the wild-type mice, respectively, demonstrating that the ability concerning fatty acid uptake in the PPARα-null mice is probably inferior to that in the wild-type mice.

Analysis of Overall Fatty Acid β-Oxidation Activity—As shown in Table I, several enzymes involved in fatty acid β-oxidation had lower constitutive expression levels in the PPARα-null mice. To evaluate the significance of the altered activity levels of fatty acid β-oxidation enzymes, overall myocardial β-oxidation activity was measured, using octanoic acid (C-8), palmitic acid (C-16), and lignoceric acid (C-24) as substrates (Fig. 3). The octanoic acid β-oxidation activity of the PPARα-null mice was lower than that of wild-type mice, which is consistent with the lower expression levels of MCAD, SCAD, and SCHAD, having higher catalytic activities for medium and short chain fatty acids, in the PPARα-null mice, respectively (Table I). The palmitic acid β-oxidation activity of the PPARα-null mice was very low, reflecting the reduced expression of long chain-specific mitochondrial fatty acid-metabolizing proteins (VLCAD, TPα, LACS, and CPT II) (Table I). Lignoceric acid β-oxidation activities of the two strains were nearly identical, reflecting the similar expression levels of very long chain-specific peroxisomal fatty acid-metabolizing proteins (acyl-CoA oxidase, peroxisomal bifunctional protein, peroxisomal thiolase, and very long chain acyl-CoA synthetase) (Table I). The overall β-oxidation activities in hearts from 32-week-old mice were similar to those from 16-week-old mice (Fig. 3), indicating the absence of age-dependent change, as expected.

Fatty Acid Metabolism in Vivo—The uptake of \(^{125}\)I-labeled compound (% dose/g tissue) in the heart is summarized in Table II. The myocardial initial uptake (at 3 min after injection) of \(^{125}\)I-9MPA was higher in wild-type than in the PPARα-null mice, suggesting that the myocardial initial uptake decreased due to lower levels of FATP and FAT in the PPARα-null mice (Fig. 2).

Although FATP was rapidly metabolized to 3MNA and only a small amount of 9MPA remained at 3 min after injection in the wild-type mice, the conversion clearly decreased in the PPARα-null mice (Table II and Fig. 4). The 3MNA/9MPA ratios in wild-type and the PPARα-null mice were 4.5 and 1.3 (at 3 min after injection) and 5.9 and 2.9 (at 10 min after injection), respectively (Table II). The slower conversion in the PPARα-null mice is compatible with the very poor myocardial β-oxidation activity of palmitic acid (Fig. 3).

Histological Analysis—The heart from wild-type mice at the age of 32 weeks seemed to be histologically normal (Fig. 5a (α and d), Table III). On the other hand, the heart from the PPARα-null mice at the age of 16 weeks showed a little focal fibrosis and myocardial degeneration associated with contraction band necrosis (Fig. 5a (b and e), Table III). At the age of 32 weeks, diffuse fibrosis occupied one-third of the inner wall of the myocardium and marked myofibrillar fragmentation of the myocardium was observed (Fig. 5a (c and f), Table III). Inflammatory infiltrates were predominantly composed of macrophages with a few lymphocytes and neutrophils. These pathological findings were not specific for any myocardial disease and were therefore regarded as an unclassified cardiomyopathy. Electron microscopy revealed that the cristae of mitochondria increased in number and density in the myocardial cells of PPARα-null mice at 16 and 32 weeks (Fig. 5b, (B and C), Table III). The number of caveolae in the cardiac capillary endothelium of PPARα-null mice (Fig. 5b, (E and F), Table III) was larger than that of wild-type mice (Fig. 5b, (D), Table III). Interestingly, the cardiac abnormalities, the myocardial fibrosis and the degeneration, appear to proceed in an age-dependent manner.

ATP, Calcium, and Magnesium Concentrations in Myocardium—To examine the relation between the impaired fatty acid catabolic ability and the cardiac abnormality in the PPARα-null mice, we carried out experiments in which mice were exposed to the stresses, starvation, and high temperature. The former stress was adopted to enhance dependence on fatty acids/triglycerides as energy sources by reducing serum glucose and lactate concentrations, and the latter to increase the load to cardiac muscle. The cardiac palmitic acid β-oxidation activity after giving these stresses slightly decreased, 5–12% lower than the constitutive activity in the wild-type mice and 2–9% lower than that in the PPARα-null mice, respectively, suggesting that these stresses weakly influenced the fatty acid catabolic ability. As shown in Table IV, starvation stress slightly reduced ATP, calcium, and magnesium concentrations, while starvation plus high temperature stress slightly increased the three concentrations in the wild-type mice, suggesting that the fatty acid catabolic ability corresponds to the changes induced by these stressors. On the other hand, the constitutive calcium concentration in the PPARα-null mice was distinctive in that it

![Fig. 1. Immunoblot analysis of selected fatty acid-metabolizing enzymes and H-FABP in wild-type (+/+ ) and PPARα-null (−/− ) mice at the age of 16 weeks.](image-url)
TABLE I

Immunoblot quantitation of cardiac fatty acid-metabolizing enzymes and fatty acid-binding protein in wild-type (+/+ ) and PPARα-null (−/− ) mice

| Protein | Localization | In heart | p value | In liver | p value |
|---------|--------------|----------|---------|----------|---------|
| VLCAD   | Mt           | 1.00     | 0.19 ± 0.01 | <0.001 | 0.32 ± 0.04 | 0.000 |
| LCAD    | Mt           | 1.00     | 1.10 ± 0.14 | 0.678 | 0.40 ± 0.21 | 0.039 |
| MCAD    | Mt           | 1.00     | 0.70 ± 0.03 | 0.009 | 1.05 ± 0.14 | 0.652 |
| SCAD    | Mt           | 1.00     | 0.34 ± 0.01 | <0.001 | 0.87 ± 0.14 | 0.248 |
| TPβ     | Mt           | 1.00     | 0.68 ± 0.05 | 0.007 | 0.98 ± 0.09 | 0.510 |
| TPγ     | Mt           | 1.00     | 0.87 ± 0.10 | 0.223 | 1.03 ± 0.07 | 1.000 |
| MH      | Mt           | 1.00     | 0.95 ± 0.04 | 0.802 | 1.12 ± 0.12 | 0.423 |
| SCHAD   | Mt           | 1.00     | 0.42 ± 0.03 | 0.008 | 3.90 ± 1.02 | 0.008 |
| T1      | Mt           | 1.00     | 0.89 ± 0.10 | 0.099 | 0.59 ± 0.02 | 0.001 |
| T2      | Mt/Cs        | 1.00     | 0.81 ± 0.06 | 0.215 | 0.98 ± 0.29 | 0.912 |
| LACS    | Mt/Ms        | 1.00     | 0.49 ± 0.04 | 0.010 | 0.42 ± 0.06 | 0.001 |
| CPT     | Mt           | 1.00     | 0.23 ± 0.01 | <0.001 | 0.90 ± 0.18 | 0.574 |
| MTE I   | Mt           | 1.00     | 0.88 ± 0.12 | 0.196 | 0.95 ± 0.07 | 0.567 |
| CTE II  | Cs           | 1.00     | 0.83 ± 0.11 | 0.080 | ND |  |
| AOX     | Ps           | 1.00     | 1.20 ± 0.07 | 0.564 | 0.98 ± 0.14 | 0.850 |
| PH      | Ps           | 1.00     | 0.92 ± 0.07 | 0.672 | 0.93 ± 0.20 | 0.653 |
| DBF     | Ps           | 1.00     | 0.77 ± 0.14 | 0.049 | 0.64 ± 0.01 | 0.001 |
| PT      | Ps           | 1.00     | 1.12 ± 0.07 | 0.705 | 1.06 ± 0.03 | 0.196 |
| VLACS   | Ps/Ms        | 1.00     | 0.95 ± 0.06 | 0.593 | 0.86 ± 0.07 | 0.087 |
| H-FABP  | Cs           | 1.00     | 0.92 ± 0.04 | 0.537 | ND |  |

a Data compare (+/+ ) with (−/− ) in heart.
b Data are from Ref. 12.
c Data compare (+/+ ) with (−/− ) in liver.
d A significant difference was found between heart and liver.

DISCUSSION

The energy substrate preference of the mammalian heart is tightly controlled during development and in response to diverse physiologic and pathophysiologic conditions (1, 2, 22–24). During the fetal period, glucose serves as the chief myocardial substrate (22, 24). Following birth, the mammalian heart switches to fatty acids as the chief energy substrate (25). Compared with glucose, fatty acids when oxidized provide more ATP per mole of substrate, albeit at the expense of increased oxygen consumption. Thus, fatty acid oxidation provides a greater capacity for energy production to meet the physiologic demands imposed on the postnatal mammalian heart. In myocardium, energy production by the decomposition of the fatty acids is carried out through a complicated system involving the uptake of free fatty acids, intracellular transport, synthesis of acyl-CoAs, and β-oxidation. It is unclear whether the factors in this system are cooperatively controlled. PPARα, known to

FIG. 2. Northern blot analysis of selected fatty acid-metabolizing enzymes and fatty acid transporters in wild-type (+/+ ) and PPARα-null (−/− ) mice at the age of 16 weeks. Representative samples from three separate mice were used. Total RNA (5.4 μg) from three representative mice from each group was electrophoresed on a denaturing gel and probed using cDNAs for VLCAD, LCAD, LACS, TPβ, FATP, FAT, and β-actin, respectively. The blots were exposed to autoradiographic film for 3 days.

was 1.5-fold that in the wild-type mouse. The starvation stress reduced the ATP concentration to 65% of the constitutive level and increased the calcium concentration 1.7-fold. The starvation plus high temperature stress further expanded these changes; the ATP concentration was 45% of the constitutive level and the calcium concentration increased 2.5-fold. Interestingly, the magnesium concentration hardly changed. These results suggest that a considerably low level of ATP was generated in the PPARα-null mouse under these stresses due to the impaired fatty acid catabolic ability, resulting in the increase of calcium concentration. The changes in ATP and calcium concentrations in the present study using the PPARα-null mice are similar to those observed in cardiac muscle under a moderate level of ischemia.

Heart Rate and Blood Pressure—Heart rate did not differ between the PPARα-null and wild-type mice at the ages of 16 weeks (540 ± 47 and 563 ± 50 beats/min) and 32 weeks (595 ± 42 and 578 ± 38 beats/min). On the other hand, systolic blood pressure was lower in PPARα-null mice than wild-type mice at the ages of 16 weeks (116 ± 15 and 134 ± 17 mmHg; not significant) and 32 weeks (98 ± 8 and 135 ± 9 mmHg; p < 0.05).
strongly control the fatty acid catabolism in liver (12), is abundant in cardiomyocytes (8). The role of PPARα in this tissue is unknown.

In the present study, we have performed various biochemical and histological analyses on the hearts of the PPARα-null and wild-type mice, and reached the following conclusions. (i) The constitutive expression levels of at least seven mitochondrial fatty acid-metabolizing proteins (VLCAD, MCAD, SCAD, SCHAD, TPα, LACS, and CPT II) depend to a large extent on the presence of PPARα, which is similar to the case of cardiac carnitine palmitoyltransferase I (26). The constitutive expression levels of the two membrane fatty acid transporters (FAT and FATP) also depend on the presence of PPARα. On the other hand, the constitutive expression levels of peroxisomal fatty acid-metabolizing proteins are independent of the presence of PPARα, as is the case in liver (12). (ii) The regulation of expression of two of the above mentioned mitochondrial proteins (VLCAD and LACS) has been observed in liver (12), but the five other proteins (MCAD, SCAD, TPα, SCHAD, and CPT II) are regulated differently in liver (12), indicating organ-specific regulation. (iii) Indeed, not all proteins relating to fatty acid catabolism are synchronistically controlled by PPARα, but many exhibit enhanced constitutive expression via PPARα. Therefore, the constitutive fatty acid catabolic ability, especially that toward long chain fatty acids, is strongly regulated by PPARα.

Taking these new findings together, it becomes clear that one of the important physiological roles of PPARα in myocardium is the constitutive maintenance of energy production using fatty acids as substrates. It is interesting that the constitutive maintenance is independent of aging at least for 16–32 weeks after birth.

In this study, we found histological abnormalities in heart, contraction band necrosis and myocardial fibrosis, only in the PPARα-null mice. These abnormalities were more serious in

![FIG. 3. Cardiac fatty acid β-oxidation in wild-type (+/+ and PPARα-null (−/−) mice. Total fatty acid β-oxidation of octanoic acid (panel A), palmitic acid (panel B), and lignoceric acid (panel C). Values are expressed as picomoles/min/mg of protein. Open bars and solid bars are from mice at the age of 16 and 32 weeks, respectively.](image)

![TABLE II

Distribution of 1251 radioactivity in heart following intravenous injection of 1251-labeled 9MPA

| Time | % dose/g tissue | 9MPA | 3MNA | PIPA | 3MNA/9MPA |
|------|----------------|------|------|------|-----------|
| min  | % % %          | % % %|      |      |           |
| Wild-type mice | 3 | 12.1 ± 1.9 | 3.7 ± 0.5 | 16.7 ± 2.2 | 4.7 ± 0.4 | 4.5/1.0 |
|      | 10 | 23.3 ± 2.5 | 2.1 ± 0.4 | 12.3 ± 2.0 | 12.9 ± 2.6 | 5.9/1.0 |
| PPARα-null mice | 3 | 7.9 ± 0.9 | 12.8 ± 2.2* | 16.5 ± 2.5 | 2.8 ± 0.3 | 1.3/1.0* |
|      | 10 | 12.0 ± 1.8* | 6.3 ± 0.9* | 18.3 ± 2.8 | 5.4 ± 0.8* | 2.9/1.0* |

* p < 0.01 between the wild-type and PPARα-null mice.

![FIG. 4. Thin-layer chromatography autoradiograms of metabolites derived from 1251-9MPA in the wild-type (+/+ and the PPARα-null (−/−) mice at 3 and 10 min after 1251-9MPA injection. The metabolites were analyzed as described under “Experimental Procedures.” Arrows indicate the positions of authentic specimens. The spot appearing at the origin corresponds to the esterified triglyceride. Front, solvent front.](image)
Azan-Mallory staining (3). Fibrosis (fibrosis was evident in the myocardium. The wild-type mouse at the age of 32 weeks (b and e) showed a little focal fibrosis. In the PPARα-null mouse at 32 weeks (c and f), diffuse dense fibrosis was evident in the myocardium. Fibrosis (arrows) is stained blue with Azan-Mallory staining (f). HE, hematoxylin-eosin stain. Original magnification, ×100. B, upper panels show mitochondria in the myocardial cells. The wild-type mouse at the age of 32 weeks (A). The PPARα-null mouse at the age of 16 weeks (B) and 32 weeks (C). Cristae increased in number and density in the PPARα-null mouse. Lower panels show caveolae in the cardiac capillary endothelial cells. The wild-type mouse at the age of 32 weeks (D). The caveolae in PPARα-null mouse at the age of 16 weeks (E) and 32 weeks (F) were more numerous than those in the wild-type mouse (D). Bar = 1 μm.

The cardiac muscle energy production system, utilizing fatty acids/triglycerides as a main fuel (25), has enormous potential to quickly respond to the rapid activity of animals; therefore, a very limited part of the system works under the constitutive condition. In the PPARα-null mice, the system may not be able to produce energy from fatty acids. Interestingly, the lower energy production ability in patients with genetic deficiencies in mitochondrial fatty acid β-oxidation is known to cause cardiac diseases such as hypertrophic cardiomyopathy, myocardial fibrosis, and massive accumulation of triglycerides (3, 38–42), which may support the occurrence of cardiac abnormalities in the present study. Furthermore, massive caveolae were observed in the heart of the PPARα-null mice (Fig. 5B). Caveolae seem to be important in regulating the cellular calcium concentration, since they have been shown to contain an inositol 1,4,5-triphosphate-sensitive calcium channel and an ATP-dependent calcium pump (43). The higher calcium concentration may increase the intensity of cardiac muscle contraction, which possibly causes another type of stress to myocardial cells, resulting in necrosis and fibrosis over a longer term.

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Cardiac Abnormality in PPARα-null Mice

Results are the means ± S.D. of four determinations. * and ** indicate p < 0.05 and p < 0.005 vs. control, respectively.

| ATP  | Calcium | Magnesium |
|------|---------|-----------|
| µmol/100 g wet weight | mg/100 g wet weight | mg/100 g wet weight |

| Wild-type mice |  |
|----------------|---|
| Control        | 227 ± 30 | 3.7 ± 0.5 | 28.5 ± 1.9 |
| Starvation     | 216 ± 19 | 3.3 ± 0.6 | 26.6 ± 2.2 |
| Starvation and high temperature | 292 ± 38 | 4.6 ± 0.7 | 29.9 ± 1.7 |

| PPARα-null mice |  |
|-----------------|---|
| Control         | 243 ± 21 | 5.4 ± 0.4 | 31.0 ± 2.8 |
| Starvation      | 157 ± 38* | 9.0 ± 1.3* | 33.3 ± 1.8 |
| Starvation and high temperature | 109 ± 22** | 13.6 ± 1.5** | 29.4 ± 1.9 |

to supply sufficient energy to myocardial cells for strife, excitement, starvation, and so on, leading to necrosis of the cells and successive fibrosis. These sudden occurrences naturally increase age-dependently, and the repair ability of damaged myocardial cells possibly decreases with aging.

Recently, PPARα was demonstrated to correlate with the expression of the redox-regulated and oxidant stress-activated transcription factor NF-κB in the spleens of aged mice (44). Furthermore, the levels of NF-κB and constitutive interleukins 6 and 12 in the spleens of aged PPARα-null mice were much higher than those of aged wild-type mice, and the level of lipid peroxidation in the livers of 48-week-old PPARα-null mice was much higher than that of the corresponding wild-type mice (45). These findings suggest a role for PPARα in the maintenance of redox balance during the aging process. The frequent fluctuation of redox balance may thereby promote age-dependent cardiac abnormality in the PPARα-null mice.

In conclusion, our present studies suggest age-dependent cardiac damage is caused by defect in the PPARα mediated signaling pathways in the mouse heart, although the extensive analysis of the damage mechanism remains to be performed. It is of interest to confirm the role for PPARα in cardiac muscle in the near future.

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