Identification of Functions of Peroxisome Proliferator-Activated Receptor α in Proximal Tubules

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Abstract. Peroxisome proliferator-activated receptor α (PPARα) is a member of the steroid/nuclear receptor superfamily that is intensively expressed in the kidney, but its physiologic function is unknown. In this study, PPARα-null mice were used to help clarify the function. Starved PPARα-null mice were found to secrete significantly more quantities of urine albumin than starved wild-type mice. Furthermore, the appearance of giant lysosomes, marked accumulation of albumin, and an impaired ability concerning albumin digestion were found only in proximal tubules of the starved PPARα-null mice. These abnormalities were probably derived from ATP insufficiency as a result of the starvation-induced decline of carbohydrate metabolism and a lack of PPARα-dependent fatty acid metabolism. It is interesting that these abnormalities disappeared when glucose was administered. Taken together, these findings demonstrate important functions of PPARα in the proximal tubules, the dynamic regulation of the protein-degradation system through maintenance of ATP homeostasis, and emphasize the importance of the fatty acid metabolism in renal physiology.

Recently, considerable attention has been paid to the peroxisome proliferator-activated receptor α (PPARα), which is known as a member of the steroid/nuclear receptor superfamily (1). According to some studies (2,3), a high level of PPARα is found in the kidney and mainly localizes in the proximal tubules, although its physiologic function has not yet been clarified. Proximal tubular epithelial cells are highly differentiated cells that reabsorb many substances that are essential to the body from glomerular filtrate and secrete several physiologically active compounds. It was suggested that ATP produced in the proximal tubular epithelial cells, which contain a greater density of mitochondria, is necessary for supporting its specific functions and maintaining basic cell functions (4).

To elucidate the physiologic role of PPARα in the kidney, we examined the reabsorption process in the proximal tubules, using PPARα-null mice. We focused on albumin reabsorption, a typical component of the filtered protein handling, done through efficient receptor-mediated endocytosis in which megalin acts as the main receptor (5–8). The relationship between albumin reabsorption and energy production was also determined, because PPARα is known to play an important role as a potent regulator of mitochondrial energy production in the liver and heart (9,10). In addition, we used starved mice to increase the dependence on fatty acids as an energy fuel source and to reduce the effect of carbohydrate metabolites.

Materials and Methods

Materials

Anti-mouse albumin IgG was purchased from Bethyl Laboratories (Montgomery, TX). Anti-rat cathepsin D IgG, ATP, acetyl-CoA, and tripalmitin were from Wako (Osaka, Japan). Anti-Rab5a IgG, anti-Rab7 IgG, and anti-mouse cathepsin L IgG were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti–LAMP-1 IgG was from American Research Products (Belmont, MA). Anti-megalin polyclonal antiserum (11) was provided by Dr. Marilyn G. Farquhar (University of California, CA), and anti-rat β-glucuronidase IgG (12) was from Dr. K. Sukegawa (Gifu University School of Medicine, Gifu, Japan).

Animals and Starvation Treatment

PPARα-null mice with an Sv/129 genetic background were produced as described previously (13). Wild-type Sv/129 were used as controls in all experiments. Two groups of mice, wild-type and PPARα-null mice, were starved for 48 h with water provided ad libitum. They were fed again for 48 h and then subjected to a second period of starvation for 48 h. After the second starvation, 150 μl of 5% and 50% glucose solution and that of 2.2% and 22% tripalmitin suspension were respectively administered to the (−/−) mice by gavage. The administration was repeated three times every 3 h.
Analysis of Urine Protein

Throughout the experiment, urine collection was carried out every day. Using the urine from the latter 24 h in each period, the urinary protein was precipitated by 20% (w/vol) TCA. After neutralization and homogenization, water was added to the original urine volume, followed by protein measurement with a BCA protein assay kit (Pierce, Rockford, IL).

Light Microscopic and Electron Microscopic Study

The preparation of light and electron microscopic sections of kidney tissues were based on the methods described previously (14). After the mice were anesthetized with ether, the kidneys were perfused with perfusate composed of 4% paraformaldehyde and with 0.1 M sodium phosphate (pH 7.4) through the left heart ventricle. For light microscopy, the tissue pieces were fixed with the same perfusate for 24 h and embedded in paraffin. The kidney sections were stained with periodic acid-Schiff reagent. For electron microscopy, the tissue pieces were fixed with fixative containing 2.5% glutaraldehyde and with 0.1 M sodium phosphate buffer (pH 7.4) for 24 h. They were osmicated, dehydrated in upgraded ethanols, and embedded in Epon resin. Semithin sections were stained with toluidine blue-O. Ultrathin sections were double stained with 1% uranyl acetate and lead citrate and examined in a JEM 1200EX II (JEOL, Tokyo, Japan) at 80 kV.

Immunohistochemical Procedures

For immunofluorescence microscopy, the preparation of sections of kidney tissues was carried out as described previously (14). Semithin cryosections were incubated with the primary antibody, followed by FITC- or Cy3-conjugated secondary antibody. The coverslips were viewed with a confocal imaging system (Fluoview; Olympus, Tokyo, Japan). For immunoelectron microscopy, the preparation of sections of kidney tissues and the labeling procedure were carried as described previously (15). The tissue pieces were fixed for 20 h in a fixative containing 4% paraformaldehyde, 0.1% glutaraldehyde, and 0.1 M sodium phosphate buffer (pH 7.4); dehydrated through graded ethanols; and embedded in Lowicryl K4M. When embedding with Lowicryl K4M, polymerization under ultraviolet light was carried out at −20°C. Ultrathin sections were incubated with the primary antibody, followed by protein A-gold–labeled secondary antibody. They were double stained with 1% uranyl acetate and lead citrate and examined in a JEM 1200EX II at 80 kV.

Immunoblot Analyses

Urine samples and renal cortex extracts were subjected to 7.5% to approximately 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The membranes were incubated with the primary antibody followed by alkaline phosphatase-conjugated secondary antibody. Immunoblotting was performed using rabbit polyclonal antibodies against mouse albumin, rat cathepsin D, β-glucuronidase, and megalin and using goat polyclonal antibody against mouse cathepsin L.

mRNA Analyses

mRNA analysis was performed by Northern blotting. Total RNA from the renal cortex was extracted, electrophoresed on 1.1 M formaldehyde-containing 1% agarose gels, and transferred to nylon membranes. The membranes were incubated with 32P-labeled cDNA probes and analyzed on a Fuji system analyzer (Fuji Photo Film, Tokyo, Japan). The cDNA probes used were for cathepsin D, cathepsin L, β-glucuronidase, megalin, and glyceraldehyde-3-phosphate dehydrogenase. The cDNA probes for cathepsin D (16), cathepsin L (17), and β-glucuronidase (18) were synthesized by reverse transcriptase–PCR. As a cDNA probe for megalin, C1B was used as described previously (6).

Palmitic Acid β-Oxidation Ability

Palmitic acid β-oxidation ability was measured as described previously (19).

Measurement of ATP and Acetyl-CoA in Renal Cortex

Renal cortex was homogenized with 12-fold volume of 0.5% (w/vol) TCA and the homogenate was centrifuged at 70,000 × g for 30 min. The supernatant fraction was injected into an HPLC (Hitachi L-6000) with UV detector (Hitachi L-4200) and Chromatointegrator (Hitachi E-2500). The analytical conditions were as follows: column, 4.0 mm diameter × 250 mm stainless steel column packed with Unicil C18 (Gasukurokogyo, Tokyo, Japan); mobile phase, 0.2 M NaH2PO4 (pH 3.0) for ATP and 5% (vol/vol) acetonitrile including 0.05% acetic acid (pH 3.0) for acetyl-CoA; flow rate, 0.8 ml/min; detection, 270 nm for ATP and 260 nm for acetyl-CoA. The retention times were 5.6 min for ATP and 2.2 min for acetyl-CoA.

Measurement of Glucose, Lactic Acid, and Pyruvic Acid in Serum

The concentration of serum glucose was determined using an autoanalyzer (Olympus AU5200). The concentrations of serum lactate and pyruvic acid were measured with a Determiner LA and Determiner PA (Kyowa Medix, Tokyo, Japan), respectively.

Results

Starvation Changes Urinary Albumin Excretion

Two groups of mice, wild-type (+/+ ) and PPARα-null (−/−), were subjected to starvation to determine whether the change in the energy fuel source would influence the excretion of albumin and total proteins in their urine. Daily urinary albumin excretion and protein excretion were quantified (Figure 1). In the control state (regular feeding), there was no difference in the daily urinary albumin or protein excretion between the (+/+ ) and the (−/−) mice. During the first starvation period, the daily urinary albumin excretion tended to increase slightly in both types of mice but returned to the control level after the mice were fed again. During the second starvation period, the increase was much greater: in the (+/+ ) mice, daily urinary albumin excretion increased 2.0-fold; in the (−/−) mice, it increased to >5.2-fold the control level. The daily urinary protein excretion also increased with starvation periods but by a much less significant amount than the albumin excretion.

Starvation Causes Morphologic and Functional Changes in Proximal Tubules

Renal histologic analyses were carried out to determine the cause of the increase in urinary albumin excretion in starved mice. Light microscopic analysis using hematoxylin and eosin and periodic acid–Schiff staining and electron microscopic analysis revealed no changes in mesangial cells, glomerular epithelial cells, foot processes, glomerular basement membranes, glomerular endothelial cells, distal tubules, or collect-
were larger than 1 μm to approximately 10 μm and occupied nearly the entire cell (Figure 2E, left). These were named “giant lysosomes.” The largest class of giant lysosomes had a volume >1,000-fold that of a normal lysosome. It was suggested that the giant lysosomes were created by the fusion of normal and smaller giant lysosomes, because they were composed of various non-standard-sized lysosome-like structures. The fusing of lysosomes and giant lysosomes occurred in many places (Figure 2E, right).

To examine proximal tubules further, we conducted immunohistochemical analyses. When the presence of albumin was examined, the vesicular structures in proximal tubules and tubular basement membrane were stained with an anti-albumin antibody. In the proximal tubules of the starved (−/−) mice, the number of vesicular structures, representing the presence of albumin, had markedly increased (Figure 3A). To determine whether the vesicular structures containing albumin were giant lysosomes, we performed an immunoelectron microscopic study. Most giant lysosomes in the starved (−/−) mice were strongly positive for albumin, whereas some endosomes in the control (−/−) mice were slightly positive (Figure 3A, bottom). The vesicular structures containing albumin, therefore, seemed to correspond to the giant lysosomes. Because the albumin accumulation observed in the giant lysosomes might be derived from abnormal lysosomal functions, we tested them for cathepsin D, one of the characteristic lysosomal enzymes. In all groups other than the starved (−/−) mice, granular structures strongly positive for cathepsin D were found equally in proximal tubules, distal tubules, and collecting ducts. In the starved (−/−) mice, these granular structures, found in distal tubules and collecting ducts, were also positive for cathepsin D, but in proximal tubules, they showed a much weaker positive reaction than those of the other groups (Figure 3B). The immunoelectron microscopic study of the giant lysosomes also revealed the presence of trace amounts of cathepsin D, although the study of normal proximal tubular lysosomes of the control (−/−) mice suggested the existence of much larger amounts of cathepsin D (Figure 3B, bottom). In addition, another analysis concerning megalin was carried out, because megalin is an essential receptor for albumin internalization in proximal tubules (7). Megalin was found on the apical surface of the epithelium, and there were no differences among the test groups (Figure 3C), suggesting a normal localization and unaltered levels of the albumin receptor in the starved (−/−) mice.

A double immunostaining analysis was performed to examine the distribution of albumin along the endocytic pathway and to characterize the giant lysosomes, in which anti-albumin antibody was used with each of the anti-Rab5a, anti-Rab7, and anti-LAMP-1 antibodies that recognized marker membrane proteins of early endosomes, late endosomes, and lysosomes, respectively. In the starved (−/−) mice, albumin spots corresponded well with LAMP-1 spots, although not with Rab5a and Rab7 spots (Figure 4), demonstrating that albumin was retarded in the degradation pathway and that giant lysosomes belonged not to the endosomal but to the lysosomal category. Therefore, the main problem seemed to exist in lysosomal...
It is interesting that many albumin spots in the control (+/+) mice corresponded well with Rab5a spots. However, there were few such correspondences in the starved (−/−) mice (Figure 4, top). This discrepancy was possibly derived from the decline in the albumin internalization efficiency in the starved (−/−) mice. To examine the changes in protein digestion ability, histologic analyses of renal tissues were performed. (A) Light micrographs of sections from (+/+)) and (−/−) mouse kidneys. The kidneys were perfused and fixed with 4% paraformaldehyde and embedded in paraffin. The kidney sections were stained with periodic acid-Schiff reagent. Con., control; St., second starvation. Bar = 50 μm. (B) Electron micrographs of sections containing glomerulus. After perfusion, tissue pieces were fixed in 2.5% glutaraldehyde, postfixed in OsO₄, dehydrated, and embedded in Epon resin before routine sectioning. Ultrathin sections were double stained with 1% uranyl acetate and lead citrate. Bar = 5 μm. (C) Light micrographs of sections containing proximal tubules. Semithin sections embedded in Epon resin were stained with toluidine blue-O. Arrows indicate many vesicular structures found in proximal tubules. Arrowhead indicates giant vesicular structure. Bar = 100 μm. (D) Electron micrographs of sections containing proximal tubules. Arrow, normal lysosome; small arrowhead, giant lysosome; large arrowhead, the largest class of giant lysosome; TL, tubular lumen. Bar = 5 μm. (E) Electron micrograph of the largest class of giant lysosomes. The largest class of giant lysosomes was observed in the proximal convoluted tubules of the starved (−/−) mice (left). Lysosomes seemed to be fusing to form giant lysosome (right). Bar in left panel = 5 μm. Bar in right panel = 0.5 μm.
in lysosomal enzymes and megalin, we conducted an immunoblot analysis and a Northern blot analysis. Only in the renal cortex of the starved (−/−) mice, cathepsin D and other characteristic lysosomal enzymes, cathepsin L and β-glucuronidase, decreased at the protein level; however, there were no changes at the transcriptional level (Figure 5). These findings suggested that these lysosomal proteins became unstable only in the renal cortex of the starved (−/−) mice. However, the analysis with megalin showed no change in any of the groups (Figure 5). The findings concerning cathepsin D and megalin are compatible with those in immunohistochemical analysis (Figure 3). Thus, albumin accumulation in the larger lysosomes was probably derived from impaired lysosomal digestion ability.

**ATP Insufficiency Causes Functional Changes in Proximal Tubules**

PPARα in liver and cardiac muscle is reported to be a potent regulator of ATP production via mitochondrial fatty acid metabolism, in which PPARα directly functions in both constitutive expression and induction of most fatty acid metabolizing enzymes, including those involved in β-oxidation (9,10). The endocytosis-degradation system requires significant amounts of ATP (20,21), so the regulation of ATP production by PPARα would be expected to have a direct role in this pathway. To clarify this hypothesis, we examined the renal ATP synthetic pathway. Initially, renal energy fuel substances were analyzed. The concentrations of serum glucose, lactic acid, and pyruvic acid markedly decreased after starvation in both the (+/+) and the (−/−) mice (Figure 6A). Starvation evidently reduces the supply of metabolites derived from the carbohydrate metabolism and raises the dependency of renal ATP production on fatty acid metabolism. In the renal cortex of the (+/+) mice, the palmitic acid β-oxidation ability was slightly lowered by starvation, but the amount of acetyl-CoA, a β-oxidation metabolite, increased by approximately 60%, and ATP levels were reduced only by 30% (Figure 6B). These findings suggest that fatty acid β-oxidation, which occurs at a lower level in the control state, increases in the starved state, resulting

![Figure 3](image-url). Immunohistochemical analyses of the proximal tubule. (A) Analyses with albumin. For immunofluorescence microscopy, semithin cryosections were incubated with rabbit anti-mouse albumin IgG, followed by FITC-conjugated anti-rabbit IgG (top and middle). Bar = 100 μm. For immunoelectron microscopy, tissue pieces were fixed with 4% paraformaldehyde and 0.1% glutaraldehyde and embedded in Lowicryl K4M at −20°C. Ultrathin sections were incubated with rabbit anti-mouse albumin IgG, followed by protein A-gold labeled anti-rabbit IgG (bottom). Arrow indicates endosome slightly positive for albumin. Arrowheads indicate giant lysosomes strongly positive for albumin. Bar = 1 μm. (B) Analyses with cathepsin D. Semithin cryosections and ultrathin sections embedded in Lowicryl K4M were incubated with rabbit anti-rat cathepsin D IgG, followed by FITC and protein A-gold labeled anti-rabbit IgG, respectively. White arrow indicates a distal tubule (top and middle). Bar = 100 μm. Black arrow indicates a normal tubular lysosome strongly positive for cathepsin D. Black arrowhead indicates a giant lysosome slightly positive for cathepsin D (bottom). Bar = 1 μm. (C) Analyses with megalin. Semithin cryosections were incubated with rabbit anti-megalin antiseraum, followed by FITC-conjugated anti-rabbit IgG. Megalin was observed on the apical surface of the epithelium. Bar = 100 μm.
Figure 4. Distribution of albumin along the endocytic pathway. A double immunofluorescence staining of cryosections from PPARα-null mouse kidneys was carried out using anti-mouse albumin IgG and anti-marker membrane protein antibody. Anti-Rab5a, anti-Rab7, and anti-LAMP-1 antibodies recognized marker membrane proteins of early endosomes, late endosomes, and lysosomes, respectively. Anti-mouse albumin IgG was visualized with Cy3-conjugated secondary antibody (red), and anti-marker membrane protein IgG was visualized with FITC-conjugated secondary antibody (green). The coverslips were viewed on a laser confocal microscopy. Arrows indicate albumin spots corresponding to Rab5a spots. Bar = 20 μm. Top panels represent single immunofluorescence staining. Bar = 20 μm.
in stimulation of energy production to maintain the amount of ATP at a relatively high level. In the (-/-) mice, palmitic acid β-oxidation ability was slightly lowered by starvation. The expression levels of very-long-chain acyl-CoA dehydrogenase, long-chain acyl-CoA dehydrogenase, and long-chain acyl-CoA synthetase, which are rate-limiting enzymes in fatty acid β-oxidation (9,22) and PPARα target genes (9,10), in the starved (-/-) mice were respectively 41%, 51%, and 52% of those in the starved (+/+). The acetyl-CoA level decreased to approximately 50%, and the ATP level decreased to 25% (Figure 6B). These findings demonstrate that β-oxidation, which was low in the control state, was neither induced nor activated by starvation, and this impaired energy production seemed to lower the amount of ATP. The collapse of the ATP homeostasis in the renal cortex of the starved (-/-) mice probably caused dysfunction in the endocytic pathway, including lysosomal protein digestion.

To ascertain the importance of ATP production in proximal tubular epithelial cells, we administered glucose or triglyceride (tripalmitin) to the starved (-/-) mice to replenish the energy-supply pathway. After the second starvation, 150% and 50% glucose solution and that of 2.2% and 22% tripalmitin suspension were respectively administered to the (-/-) mice repeatedly. The caloric value of glucose was adjusted to that of tripalmitin in each concentration. A series of analyses described previously were performed. Daily urinary albumin excretion was decreased to the control level by the 50% glucose dose (Figure 7). No changes in secretion were seen at 5% glucose and at 2.2% and 22% tripalmitin. In the renal histologic analyses, only the 50% glucose dose caused significant changes. At 3 h after the administration, the number and size of giant lysosomes were markedly reduced, and at 6 h, >60% of the area of the proximal tubules had recovered to the control state (Figure 8). A decrease in albumin accumulation and an increase in lysosomal enzymes in the proximal tubules were also observed (Figure 8). In the immunoblot analysis, only the 50% glucose dose caused the levels of the three lysosomal enzymes, cathepsin D and -L and β-glucuronidase, to recover to the control levels time-dependently (Figure 9). In the analyses of acetyl-CoA and ATP, the 50% glucose dose rapidly increased the acetyl-CoA and ATP concentrations, and the increase was saturated within 3 h in the former and at approximately 6 h in the latter (Figure 10). Excessive albumin excretion, the appearance of giant lysosomes, albumin accumulation in the proximal tubular epithelial cells, and a decline of lysosomal protein digestion possibly disappeared with the higher glucose administration, thus demonstrating the importance of ATP production in maintaining normal functions in the endocytosis-degradation system. In addition, it is important to note that the higher tripalmitin administration revealed poor effects...
of 5% glucose (116 mg/dl) mice by gavage. The glucose solution and that of 2.2% and 22% tripalmitin suspension were respectively administered to the (~/-) mice; neutral and hydrophobic, neutral and basic, and acidic amino acids were, respectively, 1.6- to approximately 2.4-fold, 1.3- to approximately 2.0-fold, 1.1- to approximately 1.4-fold, and 1.5- to approximately 2.2-fold higher than the mean values of the starved (+/+ ) mice. Sta-

cistical analysis using 10 mice demonstrated a slight difference between the two groups. Blood HCO$_3^-$ concentration of the starved (~/-) mice was 20 ± 2 mM, which was similar to that of the other three groups, suggesting a low possibility of metabolic acidosis in these mice. Serum sodium, potassium, chloride, and calcium concentrations of the starved (~/-) mice were 143 ± 1 mEq/L, 4.3 ± 0.2 mEq/L, 110 ± 2 mEq/L, and 10.5 ± 0.4 mg/dl, respectively. These concentrations were very similar to those of the other three groups: 141 to approximately 143 mEq/L, 4.0 to approximately 4.6 mEq/L, 108 to approximately 113 mEq/L, and 9.8 to approximately 10.8 mg/dl, respectively. The data suggest that these electrolytes are scarcely related to the abnormalities in proximal tubular functions.

Discussion

The findings of the present study revealed several marked changes in the renal function of the starved PPARα-null mice: an increase in daily urinary albumin excretion (Figure 1), the appearance of giant lysosomes (Figure 2), albumin accumulation in the proximal tubules (Figure 3), and a dysfunction of the lysosomal digestion ability (Figure 5). The abnormalities in the proximal tubules described above are probably related to impaired functions of the degradation system; protein transport from glomerular filtrate to early endosomes, late endosomes, and lysosomes; and digestion of reabsorbed proteins. These impaired functions in the long term possibly cause a decline in albumin removal from the glomerular filtrate, resulting in an increase in urinary albumin excretion. The proper functioning of the degradation system requires high ATP consumption to maintain the internal environment of endosomes and lysosomes under acidic conditions by endosomal and lysosomal membrane proton ATPases (20,23), which is essential for dissociating ligands (albumin, etc.) from their receptor (megalin, etc.), separating lysosomal enzymes (cathepsin D, etc.) from a mannose 6-phosphate receptor, activating and stabilizing lysosomal enzymes (24), and digesting the absorbed proteins. In addition, the proper function of the endocytosis needs ATP consumption to fuel motor proteins such as kinesin, dynein, myosin I, and so forth, which are essential for transporting vesicles (endosomes and lysosomes, etc.) along the microtubules (21,25). In the present study, starvation was found to enhance the dependence of renal ATP production on fatty acid metabolism, owing to the decrease in serum glucose and carbohydrate metabolite levels (Figure 6A) (25), the increase in the serum fatty acid levels (26), and the inhibition in the uptake of lactic acid to the proximal tubules by the increased fatty acids (27,28). However, the renal fatty acid β-oxidation ability of the PPARα-null mice, which was very low in the control state, was neither induced nor activated by starvation (Figure 6B), resulting in an acute ATP insufficiency (Figure 6B). Indeed, changes in the function of motor proteins and the ligands/receptors dissociation under ATP insufficiency are not well understood, but ATP insufficiency seems to influence strongly the dissociation, activation, and stabilization of lysosomal enzymes. A series of findings in the refeeding experiments (Figures 7 through 10) further support the impor-

Other Observations

Other evidence of abnormal proximal tubular functions was examined. Obvious glucosuria was not detected in any group. Mild generalized aminoaciduria was detected in the four starved (~/-) mice; neutral and hydrophobic, neutral and polar, basic, and acidic amino acids were, respectively, 1.6- to approximately 2.4-fold, 1.3- to approximately 2.0-fold, 1.1- to approximately 1.4-fold, and 1.5- to approximately 2.2-fold higher than the mean values of the starved (+/+ ) mice. Sta-

Figure 7. Improvement of daily urinary albumin excretion by glucose administration. After the second starvation, 150 μl of 5% and 50% glucose solution and that of 2.2% and 22% tripalmitin suspension were respectively administered to the (~/-) mice by gavage. The administration was repeated three times every 3 h. (A) Immunoblot analysis of urine albumin. See Figure 1A. Con., (~/-) control (80 μg); St., (~/-) second starvation (120 μg); Glu. (5%), administration of 5% glucose (116 μg); Glu. (50%), that of 50% glucose (92 μg); Tri. (2.2%), that of 2.2% tripalmitin (109 μg); Tri. (22%), that of 22% tripalmitin (118 μg). (B) Quantification of daily urinary albumin excretion. Solid bars show daily urinary albumin excretion. Values are presented as means ± SD (n = 5). **P < 0.01 in comparison with St.
tance of ATP production in proximal tubular epithelial cells, as described. In addition, the poor response of ATP and acetyl-CoA concentrations in the null mice by the tripalmitin administration suggested that PPARα exhibits diverse functions in vivo, stimulation/activation of the whole system concerning fatty acid uptake, transport, and degradation, by substrate supply (Figures 6B and 10). Taken together, at least one important function of PPARα in the proximal tubules—i.e., the dynamic regulation of the protein-degradation system through maintenance of the ATP homeostasis, maintaining fatty acid metab-
olism in the constitutive state, and stimulation of fatty acid consumption in the starved state—is described for the first time. This conclusion also supports the significance of the fatty acid metabolism in renal physiology.

From another point of view, it is necessary to consider the amount of albumin passing through the glomerular filtration barrier during starvation. In the case of intravenous injection of significant amounts of horseradish peroxidase, the poorly digested protein accumulated in lysosomes (29). In the present study, however, the serum albumin level in the starved (+/1) mice was nearly identical to that in the starved (+/+) mice, suggesting that the albumin pressure to glomerular filtration barrier was constant between both types of mice. In addition, the glomerular system, regulating the transcapillary traffic of albumin via a size- and charge-dependent barrier, requires no ATP consumption for its function. Moreover, glucose administration in the refeeding experiments reduced urinary albumin excretion (Figure 7), and many changes explaining this reduction were observed in proximal tubules (Figure 8). It is suggested, therefore, that the outstanding urinary albumin excretion in the starved (+/−) mice insignificantly relates to the change in glomerular filtration ability. However, it is important to suppose other possible hypotheses to explain the observed proximal tubular abnormalities. First, investigation is done concerning the influence of PPARα-mediated transcriptional regulation of principal proteins in the endocytosis-degradation system, which are irrelevant to energy homeostasis. Changes in the expression level of the principal proteins and transcriptional regulation mechanisms through PPARα are not yet known except for those observed in this study. There may be a variety of changes under the absence of PPARα, however, which seems to contribute insignificantly to the proximal tubular abnormalities for the following reasons: (1) although, changes in the protein expression level probably occur in proximal tubules of the constitutive and the glucose-administrated (+/−) mice, in which ATP levels are not low, the abnormalities do not happen and disappear, respectively; and (2) although the changes also occur in the endocytosis-degradation system of the starved (+/−) mouse livers, in which ATP levels are normal probably as a result of ATP supply through glycogen degradation and so forth, there are no abnormalities observed (unpublished observations). Second, a study was performed concerning the participation of systemic changes such as metabolic acidosis, electrolyte disturbances, and hypoglycemia. As described previously, all four mouse groups used in this study represent neither metabolic acidosis nor electrolyte abnormality, demonstrating trivial participation in the observed proximal tubular abnormalities. Severe hypoglycemia, however, was found in the starved (+/−) mouse.

Figure 9. Recovery of the amount of lysosomal proteins by glucose administration. Immunoblot analyses of lysosomal proteins after refeeding (see Figure 5A). Con., (+/−) control; St., (+/−) second starvation; Glu., administration of 50% glucose solution; Tri., that of 22% tripalmitin suspension.

Figure 10. Recovery of the acetyl-CoA and ATP levels by glucose administration. (A) Time course of acetyl-CoA level in renal cortex of the (+/−) mice after refeeding. (B) Time course of the ATP level in renal cortex of the (+/−) mice after refeeding. Solid and open circles show the level after the administration of 50% glucose and 22% tripalmitin, respectively. Values are presented as means ± SD (n = 5). **P < 0.01 in comparison with St. St., (+/−) second starvation.
possibly as a result of impaired hepatic gluconeogenic ability (Figure 6A)(26). Because proximal tubules utilize fatty acids and carbohydrate metabolites as the main fuel sources and are hardly able to use glucose directly because of the presence of trace levels of glycolytic enzymes (4), severe hypoglycemia seems to contribute only trivially to these abnormalities.

One of the outstanding morphologic changes in the present study was the appearance of giant lysosomes in proximal tubular epithelial cells (Figure 2). The giant lysosomes were probably created by the fusion of normal-sized and smaller giant lysosomes, because they were composed of various non–standard-sized lysosome-like structures and the fusion occurred in many places (Figure 2). The basic characteristics of these giant lysosomes seemed to be significantly different from those in other cases showing extremely enlarged lysosomes (30–32), demonstrating that the giant lysosomes in the present study were unique. The mechanism of emergence of the giant lysosomes is suggested as follows: immature lysosomes have a tendency to expand physically as a result of an accumulation of indigestible materials; the larger lysosomes become difficult to move on microtubules because of their increased size and mass; relatively small and mobile lysosomes then collide and clump together with the immobile larger lysosomes occluding the microtubules. In this mechanism, ATP insufficiency intensively contributes to the commencement and promotion, because lysosomal enzyme levels recover, albumin accumulation in the proximal tubular epithelial cells decreases, and the number and size of the giant lysosomes are markedly reduced in relation to the rapid increase in the ATP concentration by glucose administration (Figures 8 through 10).

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