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Zakaria El Kebbaj, Pierre Andreoletti, Driss Mountassif, Mostafa Kabine, Hervé Schohn, et al.. Differential regulation of peroxisome proliferator-activated receptor (PPAR)-alpha1 and truncated PPARalpha2 as an adaptive response to fasting in the control of hepatic peroxisomal fatty acid beta-oxidation in the hibernating mammal.. *Endocrinology*, 2009, 150 (3), pp.1192-201. 10.1210/en.2008-1394 . hal-00391027

**HAL Id: hal-00391027**

**<https://hal.science/hal-00391027>**

Submitted on 3 Jun 2009

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# Differential regulation of peroxisome proliferator-activated receptor (PPAR)- $\alpha$ 1 and truncated PPAR $\alpha$ 2 as an adaptive response to fasting in the control of hepatic peroxisomal fatty acid $\beta$ -oxidation in the hibernating mammal

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**Key words**☎Acyl-CoA oxidase 1, fasting, hibernation, jerboa, peroxisomal  $\beta$ -oxidation, PGC-1 $\alpha$ , PPAR $\alpha$ ,

**Abbreviations**☎ ACOX1, Acyl-CoA oxidase 1, CPT1, carnitine palmitoyltransferase 1, MCAD, medium chain acyl-CoA dehydrogenase, PBE, peroxisomal bifunctional enzyme, PGC-1 $\alpha$ , peroxisome proliferator-activated receptor gamma co-activator-1  $\alpha$  PMP70, peroxisomal membrane protein 70kDa, PPAR $\alpha$ , peroxisome proliferator-activated receptor  $\alpha$ , PPAR $\alpha$ 1wt, PPAR $\alpha$ 1 wild type, PPAR $\alpha$ 2tr, PPAR $\alpha$ 2 truncated.

**Abstract:**

Seasonal obesity and fasting-associated hibernation are the 2 major metabolic events governing hepatic lipid metabolism in hibernating mammals. In this process, however, the role of the nuclear receptor known as peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) has not been elucidated yet. Here we show, as in human, that jerboa (*Jaculus orientalis*) liver expresses both active wild type PPAR $\alpha$  (PPAR $\alpha$ 1wt) and truncated PPAR $\alpha$  (PPAR $\alpha$ 2tr) forms, and that the PPAR $\alpha$ 1wt/PPAR $\alpha$ 2tr ratio, which indicates the availability of active PPAR $\alpha$ 1wt, is differentially regulated during fasting-associated hibernation. Functional activation of hepatic jerboa PPAR $\alpha$ , during prehibernating and hibernating states, was demonstrated by the induction of its target genes—which encode peroxisomal proteins such as ACOX1, PMP70, and catalase—accompanied by a concomitant induction of PPAR $\alpha$  thermogenic coactivator PGC-1 $\alpha$ .

Interestingly, sustained activation of PPAR $\alpha$  by its hypolipidemic ligand, ciprofibrate, abrogates the adaptive fasting response of PPAR $\alpha$  during prehibernation and overinduces its target genes, disrupting the prehibernation fattening process. In striking contrast, during fasting-associated hibernation, jerboas exhibit preferential upregulation of hepatic peroxisomal fatty acid oxidation instead of the mitochondrial pathway, which is downregulated. Taken together, our results strongly suggest that PPAR $\alpha$  is subject to a hibernation-dependent splicing regulation in response to feeding-fasting conditions, which defines the activity of PPAR $\alpha$  and the activation of its target genes during hibernation bouts of jerboas.

## Introduction:

Numerous data have shown that efficient hepatic fatty acid oxidation depends largely on the transcriptional control of peroxisome proliferator-activated receptor (PPAR)- $\alpha$  (1-5). In fasting PPAR $\alpha$ -null mice, impairment of hepatic mitochondrial or peroxisomal fatty acid oxidation is associated with a dramatic phenotype, characterized by hypoglycemia, hypoketonemia, hypothermia, and hepatic steatosis, (6-8) resembling the fatal clinical phenotype of human inborn errors in mitochondrial fatty acid oxidation (9). The critical fasting regulatory functions of PPAR $\alpha$  are dependent on the availability of coactivator proteins such as the PPAR $\gamma$  coactivator-1 (PGC-1) family (10). Indeed, PGC-1 $\alpha$ , a cold-inducible transcriptional coactivator, plays a key role in the regulation of adaptive thermogenesis, mitochondrial biogenesis, and fatty acid metabolism (10, 11).

Jerboa is a rat-sized rodent, family dipodidea, from the subdesert highlands (1500 m) of North Africa; it is mainly a nocturnal and saltatorial herbivore, which can undergo hibernation when is faced in its biotope with critical food shortage and sub-freezing air temperature. Hibernation process is characterized by confinement of jerboa at 9-10°C in the underground plugged burrows at a depth of 30-45 cm (12). Hibernation of jerboa can be induced in the laboratory when its cooled artificially (13). After hibernation induced by food deprivation, animals are characterized by the crouched position and alternate breathing pattern of deep respiration and apnea (13).

As a model of a deep hibernator, jerboas (*Jaculus orientalis*) oscillate from seasonal obesity (due to programmed excess food uptake) to prolonged hibernation-associated fasting to food scarcity of temperate-zone winters (12, 13). Recent large transcript-profiling of the liver of hibernating golden-mantled ground squirrels, *Spermophilus lateralis*, has revealed that metabolic fasting adaptation in this hibernating species is accompanied by increased expression of peroxisomal  $\beta$ -oxidation-encoding genes (14). Fasting-triggered influx of free fatty acids from adipose tissue stimulates a switch to liver fatty acid oxidation and ketone bodies for energy production (15, 16). Despite the large influx of fatty acids in hibernating animals in response to this caloric restriction, no signs of fatty liver or hepatic injury have been observed (17).

Elsewhere, PPAR $\alpha$  is mainly expressed in tissues with high levels of lipid catabolism, such as heart and liver (2). Nevertheless, in addition to the active wild type PPAR $\alpha$ 1 form (PPAR $\alpha$ 1wt) in human and in swine, the liver expresses a truncated PPAR $\alpha$ 2 isoform (PPAR $\alpha$ 2tr). PPAR $\alpha$ 2tr lacks the ligand-binding domain, which results from a

posttranscriptional exon-skipping mechanism (18, 19), and harbors dominant-negative transcriptional repressive activity on PPAR $\alpha$ 1wt (18).

In liver, PPAR $\alpha$  governs, at the transcriptional level, the expression of genes involved in fatty acid oxidation, which encode mitochondrial, peroxisomal, and some cytochrome P450 enzymes (1-3, 5, 20). In mice, fasting-associated hepatic induction of both mitochondrial and peroxisomal fatty acids oxidation is dependent on PPAR $\alpha$  activation. Such as the peroxisomal membrane protein 70 (PMP70) and the acyl-CoA oxidase 1 (ACOX1), involved respectively in peroxisomal transport and  $\beta$ -oxidation of fatty acids (1, 3, 4, 6-8). However, the mechanisms underlying the induction of hepatic fatty acid oxidation and the involvement of PPAR $\alpha$  during seasonal obesity and fasting-associated hibernation in mammals has not been explored.

In this study, we investigated the expression of PPAR $\alpha$  and its target genes during stepwise deep hibernation of jerboas. We also examined the influence of the sustained activation of PPAR $\alpha$  by its hypolipidemic ligand, ciprofibrate, on fatty acid oxidation during the prehibernation fattening period. Our results show the presence of 2 hepatic PPAR $\alpha$  forms, wild type PPAR $\alpha$ 1 and truncated PPAR $\alpha$ 2, and their season-dependent differential regulation. Prehibernation and hibernation periods are primarily dependent on the upregulation of peroxisomal fatty acid oxidation, which is accompanied by the induction of PGC-1 $\alpha$ . Forced activation of PPAR $\alpha$  with ciprofibrate abrogates the differential regulation of PPAR $\alpha$  forms and overinduces its target genes, disrupting the prehibernation fattening process.

## Materials and Methods

### *Animals and treatments*

Young adult jerboa (*Jaculus orientalis*) between 4 and 6 months old (110–140 g body weight) were captured in the area of Engil Aït Lahcen (Boulemane region in sub-desert of eastern Morocco highland, approximate altitude 1565 meters). Animal studies were conducted in accordance with the ethic committee on Animal Use and Care of the university of Bourgogne. They were pre-acclimatized in the laboratory for 3 weeks at  $22 \pm 2$  °C with food (salad and rat chow), and water *ad libitum* (Table 1). In comparison with euthermic active animals (A), pre-hibernating and hibernating animals, were kept with food in a cold room (6 °C) for 3 weeks. This group was called the pre-hibernator group (Ph). A second group was kept under the same

conditions as Ph, except that the food contained ciprofibrate at an amount of 3 mg/kg body weight/day, and was called the ciprofibrate-treated prehibernator group (PhC). The third group was housed as Ph, and at the end of 3 weeks of the pre-hibernation period, the food was removed leading after 24–36 h to hibernation (group H) (Table 1). These animals were scarified on the fourth day of hibernation. In summary, the physiological characteristics of the hibernation were as follow: after 3 weeks of cold acclimatization + 6 °C (12, 13), the rectal temperature of jerboa was not significantly different from control ( $36 \pm 1.5$  °C). After hibernation induced by food deprivation, animals were characterized by the crouched position and alternate breathing pattern of deep respiration and apnea (12, 13). The rectal temperature dropped to  $9.8 \pm 0.7$  °C and the mean heart frequency stabilized to  $9.3 \pm 1.5$  beats/min, compared to active animals with 37 °C rectal body temperature and 300 beats/min (12, 13).

#### *Preparation of tissue homogenates*

*For activity and immunoblotting of ACOX1, PBE and catalase:* 50 to 200 mg of frozen liver tissue were homogenized in 250 mM sucrose, 2 mM EDTA, 20mM Tris-HCl buffer pH 7.5 at a ratio of 3ml/100mg of tissue. The resulting homogenate was centrifuged ( $600 \times g$ , 5 min at + 4°C) and the supernatant (post-nuclear fraction) considered as the crude extract.

*For PPAR immunoblotting:* Samples were homogenized in 25 mM (1xPBS, 1% Nonidet P-40 or Igepal CA-630, 0.5% sodium deoxycholate, 0.1 % SDS) and a cocktail of protease inhibitors (Complete; Roche, Mannheim, Germany). The homogenates were centrifuged at 15,000g for 30 min at 4°C and the supernatant used for immunoblotting experiments. Protein content was estimated with the Bio-Rad assay using bovine serum albumin (BSA) as standard.

#### *Enzymatic activities measurements:*

Palmitoyl-CoA oxidase activity was measured with the fluorimetric method described by Vamecq (1990). Briefly, the reaction mixture contained: 50 mM Tris buffer, PH 8.3, 0.75mM homovanillic acid, 4400U Peroxydase (25 U), 0.025% Triton X-100 (1.5 mg/ml BSA, 12,5 mM Aminotriazole and 50 $\mu$ M Palmitoyl-CoA. *Catalase activity* was measured according to Hildebrand et al., (21). Activity was monitored at 240nm for 2 min at the room temperature. Enzymatic activity was initiated by adding crude extract into the reaction mixture containing 500 $\mu$ l of 0.1M potassium phosphate buffer pH 7.4, 200 $\mu$ l of 75mM hydrogen peroxide in

final volume of 1 ml. The catalase specific activity was calculated using  $\epsilon$  of  $43.6 \text{ M}^{-1} \cdot \text{cm}^{-1}$  for hydrogen peroxide at 240nm.

*Medium chain Acyl-CoA Dehydrogenase (MCAD)* was measured according to Flatmark et al., (22). The reaction medium contains: 20mM Phosphate buffer pH 7.6, dichlorophenolindolphenol (DCIP) 0.005% (w/v), 100  $\mu\text{M}$  de FAD, 25 to 50  $\mu\text{g}$  of crude extract. The reaction is started by 50 $\mu\text{M}$  palmitoyl-CoA. *Carnitine Palmitoyl Transferrase 1 (CPT1)* was measured according to Bieber et al., (1972). The CPT1 activity was monitored at 412nm at 25°C. The reaction medium contains: 116 mM Tris-HCl pH8.0; 1,1 mM NaEDTA; 35  $\mu\text{M}$  Palmitoyl CoA; 0,12 mM 5,5'-dithiobis(2-nitrobenzoic acid and 1,1 mM L(-) Carnitine. The CPT1 specific activity was calculated using  $\epsilon$  of  $13600 \text{ M}^{-1} \cdot \text{cm}^{-1}$ .

### *Immunoblotting*

Crude extract proteins prepared as indicated above were separated by SDS-PAGE. Electrotransfer and immunostaining were performed as described by Towbin et al. (1979). Detection was performed using alkaline phosphatase conjugated anti rabbit IgG from goat as recommended by the supplier (Santa Cruz Biotechnology). Antibodies used were polyclonal antibody raised against respectively: rat ACOX, rat catalase, rat PBE or rat PMP70, and monoclonal antibody raised against N-terminal peptide (amino acids 4–96) of human PPAR $\alpha$  (R&D Systems).

### *RNA analysis*

Total RNAs were obtained from tissues, previously frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$ , using Trizol Reagent according to the supplier protocol (Invitrogen). Two  $\mu\text{g}$  of total RNA were reverse-transcribed into cDNA with 200 units of Moloney Murine Leukemia Virus Transcriptase (Promega) and 1  $\mu\text{g}$  of oligodT in a reaction mixture containing 50 mM Tris-HCl buffer pH 8.3, 75 mM KCl, 3 mM  $\text{MgCl}_2$ , 10 mM dithiothreitol and 20 pmol each of sense and anti-sense primers. Reverse-transcription reaction was incubated at  $48^\circ\text{C}$  for 45 min and  $94^\circ\text{C}$  for 2 min for 22 to 30 cycles. Primers used in PCR reactions were designed, from two different exonic regions with the highest nucleotides identity, after alignment of mRNA sequences of genes of interest from human, mouse and rat species. Sequences of primers and nucleotides position used to amplify ACOX1, PGC-1 $\alpha$ , PMP70, PPAR $\alpha$  or  $\beta$ -actin fragments were respectively: for ACOX1 (human, NM\_007292) sense 5'<sub>295</sub>ACCCGGACCTGCGC<sub>308</sub>3' and anti-sense 5'<sub>2272</sub>CAGAGCTTGGACTGCAGTG<sub>2254</sub>3' ;

for PGC-1 $\alpha$  (mouse, NM\_008904) sense 5'<sub>110</sub>AGCCAAGACTCTGTATGGA<sub>128</sub>3' and anti-sense 5'<sub>377</sub>TGTCCAGTGTCTCTGTGA<sub>360</sub>3'; for PMP70 (human, NM\_002858) sense 5'<sub>92</sub>TCTGCCTGCTCCACAAGC<sub>109</sub>3' and anti-sense 5'<sub>711</sub>GCCCTGAGCTCCAATTGCAC<sub>692</sub>3'; for PPAR $\alpha$  (human, NM\_001001928) sense 5'<sub>704</sub>ACGAATGCCAAGATCTGAGAAAGC<sub>727</sub>3' (Exon 6) and anti-sense 5'<sub>1050</sub>ATTCCGTGAGCTCCGTGACG<sub>1031</sub>3' (Exon 7) and for  $\beta$ -actin (mouse, NM\_007393) sense 5'<sub>535</sub>TCAGGTCATCACTATCGGCAA<sub>555</sub>3' and anti-sense 5'<sub>815</sub>CAGGTCATCACTATTGGCAA<sub>834</sub>3'.

#### *Densitometry and statistical analysis*

Quantifications and standardization were obtained using the housekeeping genes as control. GAPDH and  $\beta$ -actin proteins were used for Western blotting and  $\beta$ -actin gene for RT-PCR. Densitometry scanning was realized using the AlphaImager<sup>TM</sup> 1220 (AlphaInnotech, VWR International, Fontenay-Sous-Bois, France). Data were expressed as arbitrary units, as mean  $\pm$  standard deviations, meaning the fold activation relative to control active animals. Statistical analysis were performed using t-test and one-factor ANOVA *P* values were calculated by Student's *t* test using Statistica software. The level of significance accepted was  $P \leq 0.05$ .

## **Results:**

#### *Expression of PPAR $\alpha$ and PGC-1 $\alpha$ during the hibernation process*

Due to its important role in liver lipid metabolism, we explored the regulation of PPAR $\alpha$ , its coactivator PGC1 $\alpha$ , and their target genes during hibernation bouts. Prehibernation conditions to which jerboa are exposed in the wild were mimicked by housing jerboas for 3 weeks in a cold room at 6°C with access to food *ad libitum*. Two bands were detected by RT-PCR but only the upper one varied during the hibernation-time course; the presence of several PPAR $\alpha$  mRNA variants derived from alternative splicing has already been reported for different species, (18, 19, 23) and 2 spliced mRNA forms in liver have been identified in human, rabbit, rat, and mouse (23). Figure 1 shows that both PPAR $\alpha$ 1wt and PPAR $\alpha$ 2tr transcripts are expressed (lane 1 arrows), similar to the euthermic jerboa liver. Cold exposure of jerboas during the prehibernating period provoked a small but insignificant decrease (0.7-fold) of PPAR $\alpha$ 1wt mRNA, and almost no change of PPAR $\alpha$ 2tr mRNA.



In the same period, mRNA expression of the thermogenic PPAR $\alpha$  coactivator, PGC1 $\alpha$ , was induced 2.9-fold (Figure 1, lanes 1 and 2). This induction was accompanied by an increase in ACOX1 mRNA (2.7-fold) and, to a lesser extent, PMP70 mRNA (1.4-fold); both ACOX1 and PMP70, involved in the peroxisomal fatty acids oxydation pathway, are PPAR $\alpha$  target genes.

Food deprivation of prehibernating jerboas induced hibernation of animals 24 hours later, 4 days after which jerboas were sacrificed. During hibernation, fasting provoked a marked increase in PPAR $\alpha$ 1 mRNA levels by 3.4-fold and a strong decrease (0.3-fold) of PPAR $\alpha$ 2tr mRNA levels. In addition to PGC1 $\alpha$  mRNA levels, which were also strongly induced (4.5-fold), ACOX1 and PMP70 mRNA levels increased by 3.3- and 3.5-fold, respectively, during hibernation (Figure 1, compare lanes 1 and 3). Thus, transcriptional induction of both ACOX1 and PMP70 mRNA seems to be independent from PPAR $\alpha$ 1 mRNA induction during prehibernation period. While PGC1 $\alpha$ , a key activator of fatty acid oxidation, shows a mRNA increase at the same level as ACOX1 and PMP70 mRNA during prehibernation state.

#### *Hepatic synthesis of jerboa PPAR $\alpha$ 2 truncated protein is regulated during hibernation*

It has been demonstrated in several species, including human, that in addition to the active form of PPAR $\alpha$ 1wt (52-55 kDa), there is an additional inactive truncated form (29-31 kDa) derived from PPAR $\alpha$ 2tr mRNA translation (18, 23), which we designate PPAR $\alpha$ 2tr. Using a monoclonal antibody against an N-terminal polypeptide of human PPAR $\alpha$ , we observed that both PPAR $\alpha$ 1wt and PPAR $\alpha$ 2tr proteins are expressed in jerboa liver (Figure 2A). Truncated PPAR $\alpha$ 2 was expressed at lower levels in jerboa kidneys and absent in jerboa heart. Brain and muscle tissues expressed other PPAR $\alpha$  forms (around 45 kDa) (Figure 2A), which are probably other splice variants that have been described in other species (23).

In figure 2B, densitometric analysis of at least 3 samples from different animals allowed the calculation of both PPAR $\alpha$ 1wt/GAPDH and PPAR $\alpha$ 1wt/ GAPDH ratios for each hibernation state. Values were standardized to the ratios of active jerboas (Figure 2B). During prehibernation, the level of the 52 kDa hepatic PPAR $\alpha$ 1wt protein increased 1.87-fold (Figure 2B), compared with active jerboas. This was concomitant with a modest increase in the PPAR $\alpha$ 1wt/PPAR $\alpha$ 2tr ratio, as indicated by a 1.36-fold activation in prehibernating jerboa liver (Figure 2). During hibernation, the level of active PPAR $\alpha$ 1wt protein did not vary significantly (1.15-fold increase) (Figure 2B). Western blotting, however, revealed a nearly complete absence of truncated PPAR $\alpha$ 2 (0.06-fold), resulting in a 3.74-fold activation of the

PPAR $\alpha$ 1wt/ PPAR $\alpha$ 2tr ratio, compared with active jerboa (Figure 2B). This change indicates that the transition from the prehibernation period to the hibernation state involves a nearly 3.74-fold increase in the availability of hepatic PPAR $\alpha$ 1wt protein.

*Effect of PPAR $\alpha$  ligand ciprofibrate, a hypolipidemic agent, on the expression of PPAR $\alpha$ , its coactivator PGC1 $\alpha$ , and its target genes ACOX1 and PMP70*

In the liver of prehibernating jerboas, ciprofibrate treatment increased PPAR $\alpha$ 1wt mRNA levels by 2.8-fold, accompanied by a similar increase (3.1-fold) in PPAR $\alpha$ 2tr mRNA (Figure 1, lane 4). This PPAR $\alpha$  ligand induced PGC-1 $\alpha$  mRNA 1.8-fold, compared with euthermic jerboas. Ciprofibrate treatment also increased ACOX1 and PMP70 mRNA expression 20.6- and 4.8-fold, respectively (Figure 1, lane 4). At the protein level, immunoblotting revealed a slight increase of PPAR $\alpha$ 1wt and PPAR $\alpha$ 2tr (Figure 2B). The calculated PPAR $\alpha$ 1wt/PPAR $\alpha$ 2tr ratio, however, was decreased by ciprofibrate to 0.69-fold, compared with a 1.36-fold activation observed in prehibernating control animals, suggesting that the livers of prehibernating ciprofibrate-treated jerboas possess an increased amount of truncated PPAR $\alpha$ 2 and 69% less available PPAR $\alpha$ 1wt protein.

*Peroxisomal  $\beta$ -oxidation system enzymes, PPAR $\alpha$  target genes in hibernating jerboa*

Peroxisomal acyl-CoA  $\beta$ -oxidation begins with ACOX1, the starting enzyme, and PBE as the second enzyme of this pathway. ACOX1 releases enoyl-CoA and also H<sub>2</sub>O<sub>2</sub> as a byproduct, which is a highly oxidant molecule that is degraded rapidly by catalase, a peroxisomal antioxidant protein. PMP70 is involved in the metabolic transport of fatty acids into peroxisomes (24). The expression of these 4 peroxisomal enzymes has been shown previously to be dependant on PPAR $\alpha$  (1, 5). In addition, the 72 kDa ACOX1 protein (polypeptide A) undergoes a unique posttranslational proteolytic cleavage responsible for the appearance of 2 additional bands of 50 and 20.8 kDa, respectively, called polypeptides B and C.

Cold exposure during the prehibernation period slightly increased the level of peroxisomal proteins involved in  $\beta$ -oxidation—ACOX1, PBE, and PMP70—above 1.4-fold, whereas only catalase protein was induced 1.9-fold (Figure 3, lane Ph). This increase in peroxisomal proteins was accompanied by both a 40% increase (1.4-fold) in peroxisomal palmitoyl-CoA oxidation and a 100% increase in catalase activity (2.1-fold) (Figure 4A, lane Ph).

The hibernation period was characterized by a strong induction of peroxisomal proteins: ACOX1, 3-fold; PBE, 4.2-fold; and catalase, 3.5-fold. Only PMP70 showed a modest

variation of 1.2-fold (Figure 3, lane H). In addition, peroxisomal palmitoyl-CoA oxidation increased by 180% (2.8-fold), and catalase activity increased by 50% (1.5-fold) (figure 4A). This underline that the peroxysomal fatty acid oxidation pathway is differentially regulated during respectively prehibernation and hibernation periods.

#### *Hibernation-dependent depression of mitochondrial fatty acid $\beta$ -oxidation*

The level of mitochondrial fatty acid oxidation is controlled by carnitine palmitoyl-CoA transferase (CPT1) activity, which is the rate-limiting enzyme responsible for the entry of long-chain fatty acids into the mitochondria (25). During prehibernation, liver CPT1 activity was significantly decreased by 26% (0.74-fold) (Figure 4B, lane Ph), accompanied by a 43% decrease (0.57-fold) of the activity of mitochondrial medium chain acyl-CoA dehydrogenase (MCAD) (Figure 4B, lane Ph). Starvation-induced hibernation was also associated with a 30% depression of CPT1 activity (0.70-fold) (Figure 4B, lane H), compared with that of active euthermic jerboas. But with regard to the prehibernation period, mitochondrial MCAD activity increased greater than 30% (0.57-fold during prehibernation and 0.86-fold during hibernation) (Figure 4B, lane Ph and H). By contrast to peroxisomes mitochondria which exhibits a higher capacity of fatty acid oxidation, seems to be negatively regulated during the hibernation bouts.

#### *Effect of ciprofibrate on mitochondrial and peroxisomal $\beta$ -oxidation, and peroxisome proliferation*

In rodents, induction of peroxisomal  $\beta$ -oxidation by ciprofibrate has been shown to be a PPAR $\alpha$ -dependent phenomenon, which is accompanied by peroxisome proliferation (i.e., an increase in the number and size of peroxisomes) (1, 5, 26). ACOX1, PBE, or PMP70 are typical PPAR $\alpha$ -inducible biomarkers of both peroxisomal  $\beta$ -oxidation and peroxisome proliferation (5). In the preceding section (Figure 1, lane PhC), we demonstrated a strong increase of hepatic ACOX1 mRNA in prehibernating ciprofibrate-treated jerboas. As shown in figure 4, lane PhC, immunoblotting revealed a remarkable induction of peroxisomal  $\beta$ -oxidation enzymes: ACOX1, 6.2-fold; PBE, 5.6-fold; and catalase, 5.6-fold. PMP70 was not strongly induced (1.2-fold). Such inductions were accompanied by a strong increase in palmitoyl-CoA oxidase activity of ACOX1 by 8.2-fold and catalase activity by 2.9-fold (Figure 4A).

In contrast to peroxisomal fatty acid  $\beta$ -oxidation, mitochondrial CPT1 activity was slightly decreased (0.82-fold), compared with the activity in euthermic jerboas; comparison with prehibernating untreated jerboas, however, revealed no significant effect of ciprofibrate (Figure 4B, lane PhC). MCAD activity decreased by 25% (0.75-fold), and compared with prehibernating control jerboas (0.57-fold), MCAD activity increased by 18% (0.75-fold) in the liver of prehibernating jerboas treated with ciprofibrate (Figure 4B, lane PhC). Thus, the overinduction of peroxisomal fatty acids oxidation during the prehibernation period by hypolipidemic drug, such as ciprofibrate, prevents fat accumulation in the prehibernating jerboas required for the energy supply during hibernation period.

## Discussion:

Seasonal obesity is an evolutionary strategy adopted by hibernators to anticipate food scarcity in inhospitable environments and to control energy consumption in a strict manner. Here, we report a stepwise deep hibernation of jerboas under laboratory conditions mimicking those in the wild (12). Before entering hibernation, jerboas need 3 weeks of cold acclimatization (prehibernation period) with food provided *ad libitum*. Starvation-induced hibernation in jerboas is characterized by a dramatically lowered heartbeat rate, as well as hypothermia to 9°C. During prehibernation, these parameters, particularly body temperature, remain similar to those of active animals, despite the lower ambient temperature (6°C).

During autumn, corresponding to the prehibernation period, jerboas have to manage 2 apparent contradictory demands: (1) fat storage in the adipose tissue as energetic fuel for the hibernation period, and (2) immediate energy supply in a cold prehibernation environment, necessary for thermogenic acclimatization and maintenance of a body temperature of 37°C.

Our previous studies have shown that prehibernation of jerboas is associated with hypotriglyceridemia and hypoketonemia, which result from a high rate of fatty acid and triacylglycerol uptake concomitant with depressed mitochondrial and peroxisomal fatty acid oxidation in adipose tissues (27, 28). We have also reported the participation of jerboa PPAR isotypes  $\beta/\delta$  and  $\gamma$  in brown fat non-shivering thermogenesis and in white fat adipogenesis during hibernation bouts, respectively (28). As in other hibernators, carbohydrates are the main source of energy in prehibernating jerboas, which exhibit the same normal circulating glucose levels as euthermic animals (28). Carbohydrate use helps animals preserve optimal energetic fat storage for their hibernation period (15, 29).

Here, we show that the expression of PPAR $\alpha$  in prehibernating jerboa liver is regulated at different levels. Two alternatively-spliced transcripts of PPAR $\alpha$  and 2 forms, PPAR $\alpha$ 1wt (52 kDa) and PPAR $\alpha$ 2tr (29 kDa), are primarily expressed in the jerboa liver and, to a lesser extent, in kidney. Such a tissue-specific expression profile in jerboas is similar to the human expression pattern of PPAR $\alpha$  isoforms (18). The truncated PPAR $\alpha$ 2 protein lacks the ligand-binding domain (18). PPAR $\alpha$ 1 mRNA expression is not changed significantly during prehibernation, while at the protein level, the PPAR $\alpha$ 1wt/PPAR $\alpha$ 2tr ratio is slightly increased. This seems to be sufficient for the induction of PPAR $\alpha$  target genes implicated in peroxisomal fatty acid oxidation (i.e. ACOX1 and PBE) and the recruitment of the coactivator PGC1 $\alpha$ , which is clearly upregulated during prehibernation in the jerboa liver.

Concomitant induction of ACOX1, PBE, and PGC1 $\alpha$  is consistent with the known key role of PGC1 $\alpha$  in cold-induced fatty acid oxidation and PPAR $\alpha$  coactivation (30). Transcriptional activation of the ACOX1 and PBE genes throughout PGC1 $\alpha$  coactivation by other nuclear receptors (e.g. hepatic nuclear factor-4 or FOXO1) is not excluded; hepatic nuclear factor-4 has a shared ability with PPAR $\alpha$  to bind PPAR $\alpha$  response elements of the ACOX1 and PBE genes (31, 32), and FOXO1 expression has been shown to be upregulated in the hibernating ground squirrel (*Spemophilus tridecemlineatus*) (33).

The substantial increase in PPAR $\alpha$  target genes (i.e. ACOX1, PBE, PMP70, and catalase) during the prehibernation fattening period is surprising. But, we have to consider that ACOX1, an oxidoreductase, also catalyzes the ultimate anabolic step of docosahexanoic acid synthesis, an important polyunsaturated fatty acid for normal brain function (34, 35). Thus, PPAR $\alpha$ -dependent induction of the peroxisomal  $\beta$ -oxidation pathway may anticipate the selective retention of essential polyunsaturated fatty acids during the hibernation process (36). Furthermore, that the limited hepatic peroxisomal degradation of fatty acids is induced during the fattening prehibernation period may prevent the jerboa from developing a hepatic steatosis (37). Indeed, ACOX1 $^{-/-}$  knockout mice develop hepatic steatosis accompanied by sustained activation of hepatic PPAR $\alpha$  and its target genes (7), which underscores the metabolic crosstalk between the nuclear receptor PPAR $\alpha$  and its target gene, ACOX1 (5). In prehibernating conditions, jerboa ACOX1 plays an additional role as a PPAR $\alpha$  sensor, most likely by metabolizing the PPAR $\alpha$  acyl-CoA-ligand.

Long chain fatty acids are proposed to regulate CPT1, a rate-limiting enzyme of mitochondrial fatty acid oxidation, in a PPAR $\alpha$ -independent manner (38). Thus, decreased CPT1 activity in jerboa liver during prehibernation is probably due to the lipogenesis-associated increase in liver malonyl-CoA concentrations, which in turn block fatty acid transport across the mitochondrial membrane. This is a consequence of the malonyl-CoA allosteric inhibition of CPT1 (25). Consequently, mitochondrial MCAD fatty acid oxidation, as we reveal in this study, is also decreased in the liver of prehibernating jerboas. Thus, the decrease in mitochondrial fatty acid oxidation is required during this fattening period of prehibernation.

Here, we report that the hibernation-associated fasting period in jerboa liver is characterized by a differential induction of PPAR $\alpha$ 1wt transcripts with a near absence of PPAR $\alpha$ 2tr mRNA. Numerous data from several groups using PPAR $\alpha$ -null mice have demonstrated the crucial role of PPAR $\alpha$  in the adaptive response to fasting and the regulation of liver fatty acid

oxidation rates (4, 8, 39, 40). But the physiological significance of the expression of PPAR $\alpha$  isoforms in liver, particularly PPAR $\alpha$ 2tr, has not been elucidated yet.

In the present study, we show for the first time the physiological link between hibernation-associated fasting in the jerboa liver and the upregulation of the ratio of active PPAR $\alpha$ 1wt to truncated PPAR $\alpha$ 2 isoforms as a response to this metabolic challenge. Posttranscriptional regulation by alternative splicing is a general mechanism that has been described for other nuclear receptors, such as the glucocorticoid receptor (41). A strong diminution of the expression of the dominant-negative PPAR $\alpha$ 2tr in the hibernating jerboa liver has an immediate consequence on the availability of active PPAR $\alpha$ 1wt. Through endogenous activation of PPAR $\alpha$ 1wt, this triggers the upregulation of peroxisomal  $\beta$ -oxidation enzyme-encoding genes—such as ACOX1 and L-PBE, which are PPAR $\alpha$  target genes (42, 43). Such induction of PPAR $\alpha$ -dependent fatty acid oxidation is accompanied by the induction of the thermogenic PPAR $\alpha$  coactivator, PGC-1 $\alpha$ . In addition, recent studies have reported the increase in PGC-1 $\alpha$  levels in the liver of hibernating bats (*Myotis lucifugus*) (44) and in heart and muscle tissue of hibernating ground squirrels (*Spermophilus tridecemlineatus*) (45).

The relative preference of the induction of peroxisomal fatty acid  $\beta$ -oxidation instead of the mitochondrial pathway can be interpreted in several ways. During hibernation, jerboa body temperature is above 10°C, which is associated with a slow metabolic rate concomitant with the inhibition of carbohydrate oxidation and the induction of a specific pancreatic lipase that releases fatty acids from triglycerides at temperatures as low as 0°C (29, 46). In this context, the 180% increase of peroxisomal  $\beta$ -oxidation has to be considered, in the absence of coupling to oxidative phosphorylation, as a thermogenic source for jerboa liver. On the other hand, induction of peroxisomal fatty acid oxidation, through PPAR $\alpha$ 1wt activation, may underlie the anabolic side of peroxisomes in the synthesis of polyunsaturated fatty acids, which has been well documented (47, 48).

Furthermore, specific lipids, as essential fatty acids and their degree of insaturation, play a determinant role in the frequency of interbouts, in which animals arouse from hibernation during winter (49). Deficiency of such essential fatty acids implicates an increase in the frequency of arousing and rewarming. Consequently, this increase in energy expenditure impairs the ability of hibernators to survive through long winter seasons. Surprisingly, the increase in PPAR $\alpha$ 1 mRNA is not accompanied by an expected augmentation in PPAR $\alpha$ 1wt protein. This may be explained by the regulated shutdown of cellular protein synthesis throughout the phosphorylation of elongation factor-2 during hibernation (50). Due to its key

role in fatty acid oxidation, such high levels of PPAR $\alpha$ 1wt mRNA may promote synthesis of the active PPAR $\alpha$ 1wt form during arousal. Indeed, throughout the winter period, jerboas arouse periodically (51), and 90% of the estimated energetic expenditure throughout winter is used during arousal (52).

In previous studies, we have shown ciprofibrate-dependent peroxisome proliferation and induction of peroxisomal fatty acid oxidation in euthermic active jerboa liver (13, 26). In addition, another fibrate molecule, clofibrate, has been shown to induce PPAR $\alpha$  in euthermic jerboa tissue (53).

Because of its properties as a hypolipidemic drug and a well-known PPAR $\alpha$  ligand, ciprofibrate was administrated to a group of prehibernating jerboas. We observed the absence of fat accumulation in treated jerboas, compared with prehibernating untreated animals (28). In jerboa liver, the induction of both PPAR $\alpha$  mRNA splice variants,  $\alpha$ 1 and  $\alpha$ 2, was accompanied by a strong increase of PPAR $\alpha$  target genes—ACOX1, L-PBE, PMP70, and catalase—leading to high fatty acid degradation and less fat storage in adipose tissue.

At the protein level, increases in the hepatic PPAR $\alpha$ 2tr form may have consequences on the transcriptional activity of other nuclear receptors in jerboas. Indeed, it has been shown that human PPAR $\alpha$ 2tr exerts transcriptional repressive activity as a dominant negative receptor on PPAR $\alpha$ 1wt, PPAR $\gamma$ , hepatic nuclear factor-4, and glucocorticoid receptor by titration of a shared common coactivator, CREB-binding protein/p300 (18). Disturbance of lipid metabolism in jerboas by a hypolipidemic drug resulted in the death of all animals within 24 h after the induction of hibernation by food withdrawal. Thus, during jerboa prehibernation, induction of both PPAR  $\alpha$ 1wt and  $\alpha$ 2tr forms in the liver by ciprofibrate effects fatal consequences by enhancing unexpected fatty acid oxidation and by disrupting the nuclear receptor–coactivator crosstalk required for transition to the hibernating state.

Regarding the lipid homeostasis regulation, alternative splicing and has been described for other transcription factors such as sterol regulatory element-binding proteins (SERBPs). SERBP-1a isoform is a potent activator of both cholesterol and fatty acid biosynthetic pathways, while SERBP-1c isoform derived from the same gene is mainly specific for the fatty acid biosynthesis (54). On the other hand, the overexpression of PPAR $\gamma$ 1 splice isoform is sufficient to induce adipocyte-specific genes in mouse liver (55). In addition, in human, the caloric restriction-associated regulation of uncoupling protein-3, a mitochondrial thermogenic protein, is dependent on the alternative splicing mechanism in skeletal muscle (56). In this context, our results on the differential expression of the wild type (PPAR $\alpha$ 1wt) and the



truncated (PPAR $\alpha$ 2tr) isoforms reveal that the alternative transcription regulation is part of a general mechanism which impacts in hibernating jerboa the adaptive response to fasting in the control of hepatic peroxisomal fatty acid  $\beta$ -oxidation.

In conclusion, the data presented here, using an original model of a small, true-hibernating rodent (jerboa), revealed that differential expression of PPAR isoforms  $\alpha$ 1wt and  $\alpha$ 2tr participates in the control of hepatic fatty acid oxidation during hibernation of jerboas as an adaptive response to fasting. Therefore, we proposed a model of the metabolic regulation of PPAR $\alpha$  target genes. In such model, the activity of the liver pool of PPAR $\alpha$  depends largely on the balance of the ratio between the wild type PPAR $\alpha$ 1wt and the dominant negative truncated PPAR  $\alpha$ 2tr forms during hibernation bouts (Figure 5). A preferential upregulation of peroxisomal fatty acid oxidation is essential for jerboas during both prehibernating and hibernating periods, which are accompanied by the induction of hepatic PGC-1 $\alpha$ . Taken together, our results strongly suggest that PPAR $\alpha$  is subject to regulation of hibernation-dependent splicing in response to fasting conditions, which define the activity of PPAR $\alpha$  and subsequently the activation of its target genes during hibernation bouts of jerboas.

## Acknowledgments

This work has been supported by the Regional Council of Burgundy and IFR100, by the Morocco “programme d’appui à la recherche scientifique, Biologie no134”, by the action intégrée franco-marocaine CMIFM-AI-MA/05/134 of the PAI *volubilis* program, Ministère des Affaires Etrangères and by the Institut National de la Santé et de la Recherche Médicale. This project were supported by the European Union project “‘Peroxisome’” LSHG-CT-2004–512018.

## References

1. **Cherkaoui-Malki M, Meyer K, Cao WQ, Latruffe N, Yeldandi AV, Rao MS, Bradfield CA, Reddy JK** 2001 Identification of novel peroxisome proliferator-activated receptor alpha (PPARalpha) target genes in mouse liver using cDNA microarray analysis. *Gene Expr* 9:291-304
2. **Desvergne B, Michalik L, Wahli W** 2006 Transcriptional regulation of metabolism. *Physiol Rev* 86:465-514
3. **Lee SS, Pineau T, Drago J, Lee EJ, Owens JW, Kroetz DL, Fernandez-Salguero PM, Westphal H, Gonzalez FJ** 1995 Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol Cell Biol* 15:3012-22
4. **Leone TC, Weinheimer CJ, Kelly DP** 1999 A critical role for the peroxisome proliferator-activated receptor alpha (PPARalpha) in the cellular fasting response: the PPARalpha-null mouse as a model of fatty acid oxidation disorders. *Proc Natl Acad Sci U S A* 96:7473-8
5. **Reddy JK** 2004 Peroxisome proliferators and peroxisome proliferator-activated receptor alpha: biotic and xenobiotic sensing. *Am J Pathol* 164:2305-21
6. **Costet P, Legendre C, More J, Edgar A, Galtier P, Pineau T** 1998 Peroxisome proliferator-activated receptor alpha-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. *J Biol Chem* 273:29577-85
7. **Hashimoto T, Fujita T, Usuda N, Cook W, Qi C, Peters JM, Gonzalez FJ, Yeldandi AV, Rao MS, Reddy JK** 1999 Peroxisomal and mitochondrial fatty acid beta-oxidation in mice nullizygous for both peroxisome proliferator-activated receptor alpha and peroxisomal fatty acyl-CoA oxidase. Genotype correlation with fatty liver phenotype. *J Biol Chem* 274:19228-36
8. **Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W** 1999 Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. *J Clin Invest* 103:1489-98
9. **Kelly DP, Strauss AW** 1994 Inherited cardiomyopathies. *N Engl J Med* 330:913-9
10. **Finck BN, Kelly DP** 2006 PGC-1 coactivators: inducible regulators of energy metabolism in health and disease. *J Clin Invest* 116:615-22
11. **Rosen ED, Spiegelman BM** 2006 Adipocytes as regulators of energy balance and glucose homeostasis. *Nature* 444:847-53
12. **El Hilali M, Veillat JP** 1975 *Jaculus orientalis*: a true hibernator. *Mammamia* 39:401-404

13. **Kabine M, Clemencet MC, Bride J, El Kebbaj MS, Latruffe N, Cherkaoui-Malki M** 2003 Changes of peroxisomal fatty acid metabolism during cold acclimatization in hibernating jerboa (*Jaculus orientalis*). *Biochimie* 85:707-14
14. **Williams DR, Epperson LE, Li W, Hughes MA, Taylor R, Rogers J, Martin SL, Cossins AR, Gracey AY** 2005 Seasonally hibernating phenotype assessed through transcript screening. *Physiol Genomics* 24:13-22
15. **Andrews MT** 2007 Advances in molecular biology of hibernation in mammals. *Bioessays* 29:431-40
16. **Carey HV, Andrews MT, Martin SL** 2003 Mammalian hibernation: cellular and molecular responses to depressed metabolism and low temperature. *Physiol Rev* 83:1153-81
17. **Lindell SL, Klahn SL, Piazza TM, Mangino MJ, Torrealba JR, Southard JH, Carey HV** 2005 Natural resistance to liver cold ischemia-reperfusion injury associated with the hibernation phenotype. *Am J Physiol Gastrointest Liver Physiol* 288:G473-80
18. **Gervois P, Torra IP, Chinetti G, Grotzinger T, Dubois G, Fruchart JC, Fruchart-Najib J, Leitersdorf E, Staels B** 1999 A truncated human peroxisome proliferator-activated receptor alpha splice variant with dominant negative activity. *Mol Endocrinol* 13:1535-49
19. **Sundvold H, Grindflek E, Lien S** 2001 Tissue distribution of porcine peroxisome proliferator-activated receptor alpha: detection of an alternatively spliced mRNA. *Gene* 273:105-13
20. **Gulick T, Cresci S, Caira T, Moore DD, Kelly DP** 1994 The peroxisome proliferator-activated receptor regulates mitochondrial fatty acid oxidative enzyme gene expression. *Proc Natl Acad Sci U S A* 91:11012-6
21. **Hildebrand DF, Rodriguez JG, Brown GC, Luu KT, Volden CS** 1986 Peroxidative responses of leaves in two soybean genotypes injured by twospotted spider mites (Acari: Tetranychidae). *J Econ Entomol* 79:1459-1465
22. **Flatmark T, Ruzicka FJ, Beinert H** 1976 The pattern of iron--sulfur centers in brown adipose tissue mitochondria: preponderance of ETF dehydrogenase and invariance with the thermogenic state. *FEBS Lett* 63:51-5
23. **Hanselman JC, Vartanian MA, Koester BP, Gray SA, Essenburg AD, Rea TJ, Bisgaier CL, Pape ME** 2001 Expression of the mRNA encoding truncated PPAR alpha does not correlate with hepatic insensitivity to peroxisome proliferators. *Mol Cell Biochem* 217:91-7
24. **Imanaka T, Aihara K, Takano T, Yamashita A, Sato R, Suzuki Y, Yokota S, Osumi T** 1999 Characterization of the 70-kDa peroxisomal membrane protein, an ATP binding cassette transporter. *J Biol Chem* 274:11968-76
25. **Grantham BD, Zammit VA** 1986 Restoration of the properties of carnitine palmitoyltransferase I in liver mitochondria during re-feeding of starved rats. *Biochem J* 239:485-8
26. **el Kebbaj MS, Cherkaoui Malki M, Latruffe N** 1996 Properties of peroxisomes from jerboa (*Jaculus orientalis*). *Eur J Cell Biol* 70:150-6
27. **Kabine M, Cherkaoui Malki M, Clemencet MC, El Kebbaj MS, Latruffe N** 1998 Peroxisomal changes during hibernation of Jerboa (*Jaculus orientalis*). *J Am Oil Chem Soc* 75:275-280
28. **Kabine M, El Kebbaj Z, Oaxaca-Castillo D, Clemencet MC, El Kebbaj MS, Latruffe N, Cherkaoui-Malki M** 2004 Peroxisome proliferator-activated receptors as regulators of lipid metabolism; tissue differential expression in adipose tissues during

- cold acclimatization and hibernation of jerboa (*Jaculus orientalis*). *Biochimie* 86:763-70
29. **Andrews MT, Squire TL, Bowen CM, Rollins MB** 1998 Low-temperature carbon utilization is regulated by novel gene activity in the heart of a hibernating mammal. *Proc Natl Acad Sci U S A* 95:8392-7
  30. **Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM** 1998 A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* 92:829-39
  31. **Nicolas-Frances V, Dasari VK, Abruzzi E, Osumi T, Latruffe N** 2000 The peroxisome proliferator response element (PPRE) present at positions -681/-669 in the rat liver 3-ketoacyl-CoA thiolase B gene functionally interacts differently with PPARalpha and HNF-4. *Biochem Biophys Res Commun* 269:347-51
  32. **Winrow CJ, Marcus SL, Miyata KS, Zhang B, Capone JP, Rachubinski RA** 1994 Transactivation of the peroxisome proliferator-activated receptor is differentially modulated by hepatocyte nuclear factor-4. *Gene Expr* 4:53-62
  33. **Cai D, McCarron RM, Yu EZ, Li Y, Hallenbeck J** 2004 Akt phosphorylation and kinase activity are down-regulated during hibernation in the 13-lined ground squirrel. *Brain Res* 1014:14-21
  34. **Ferdinandusse S, Denis S, Mooijer PA, Zhang Z, Reddy JK, Spector AA, Wanders RJ** 2001 Identification of the peroxisomal beta-oxidation enzymes involved in the biosynthesis of docosahexaenoic acid. *J Lipid Res* 42:1987-95
  35. **Su HM, Moser AB, Moser HW, Watkins PA** 2001 Peroxisomal straight-chain Acyl-CoA oxidase and D-bifunctional protein are essential for the retroconversion step in docosahexaenoic acid synthesis. *J Biol Chem* 276:38115-20
  36. **Xia T, Mostafa N, Bhat BG, Florant GL, Coleman RA** 1993 Selective retention of essential fatty acids: the role of hepatic monoacylglycerol acyltransferase. *Am J Physiol* 265:R414-9
  37. **Patsouris D, Reddy JK, Muller M, Kersten S** 2006 Peroxisome proliferator-activated receptor alpha mediates the effects of high-fat diet on hepatic gene expression. *Endocrinology* 147:1508-16
  38. **Louet JF, Chatelain F, Decaux JF, Park EA, Kohl C, Pineau T, Girard J, Pegorier JP** 2001 Long-chain fatty acids regulate liver carnitine palmitoyltransferase I gene (L-CPT I) expression through a peroxisome-proliferator-activated receptor alpha (PPARalpha)-independent pathway. *Biochem J* 354:189-97
  39. **Le May C, Pineau T, Bigot K, Kohl C, Girard J, Pegorier JP** 2000 Reduced hepatic fatty acid oxidation in fasting PPARalpha null mice is due to impaired mitochondrial hydroxymethylglutaryl-CoA synthase gene expression. *FEBS Lett* 475:163-6
  40. **Lemberger T, Saladin R, Vazquez M, Assimacopoulos F, Staels B, Desvergne B, Wahli W, Auwerx J** 1996 Expression of the peroxisome proliferator-activated receptor alpha gene is stimulated by stress and follows a diurnal rhythm. *J Biol Chem* 271:1764-9
  41. **Lu NZ, Cidlowski JA** 2006 Glucocorticoid receptor isoforms generate transcription specificity. *Trends Cell Biol* 16:301-7
  42. **Bardot O, Aldridge TC, Latruffe N, Green S** 1993 PPAR-RXR heterodimer activates a peroxisome proliferator response element upstream of the bifunctional enzyme gene. *Biochem Biophys Res Commun* 192:37-45
  43. **Tugwood JD, Issemann I, Anderson RG, Bundell KR, McPheat WL, Green S** 1992 The mouse peroxisome proliferator activated receptor recognizes a response

- element in the 5' flanking sequence of the rat acyl CoA oxidase gene. *Embo J* 11:433-9
44. **Eddy SF, Storey KB** 2003 Differential expression of Akt, PPARgamma, and PGC-1 during hibernation in bats. *Biochem Cell Biol* 81:269-74
  45. **Eddy SF, Morin P, Jr., Storey KB** 2005 Cloning and expression of PPAR-gamma and PGC-1alpha from the hibernating ground squirrel, *Spermophilus tridecemlineatus*. *Mol Cell Biochem* 269:175-82
  46. **Andrews MT** 2004 Genes controlling the metabolic switch in hibernating mammals. *Biochem Soc Trans* 32:1021-4
  47. **Li Y, Nara TY, Nakamura MT** 2005 Peroxisome proliferator-activated receptor alpha is required for feedback regulation of highly unsaturated fatty acid synthesis. *J Lipid Res* 46:2432-40
  48. **Sprecher H** 2002 The roles of anabolic and catabolic reactions in the synthesis and recycling of polyunsaturated fatty acids. *Prostaglandins Leukot Essent Fatty Acids* 67:79-83
  49. **Florant GL, Hester L, Ameenuddin S, Rintoul DA** 1993 The effect of a low essential fatty acid diet on hibernation in marmots. *Am J Physiol* 264:R747-53
  50. **Chen Y, Matsushita M, Nairn AC, Damuni Z, Cai D, Frerichs KU, Hallenbeck JM** 2001 Mechanisms for increased levels of phosphorylation of elongation factor-2 during hibernation in ground squirrels. *Biochemistry* 40:11565-70
  51. **Hooper ET, el-Hilali M** 1972 Temperature regulation and habits in two species of jerboa, genus *Jaculus*. *J Mammal* 53:574-93
  52. **Wang P, Walter RD, Bhat BG, Florant GL, Coleman RA** 1997 Seasonal changes in enzymes of lipogenesis and triacylglycerol synthesis in the golden-mantled ground squirrel (*Spermophilus lateralis*). *Comp Biochem Physiol B Biochem Mol Biol* 118:261-7
  53. **Amsaguine-Safir S, Bianchi A, Collet P, Huin-Schohn C, Jeanvoine A, Becuwe P, Kremarik-Bouillaud P, Domenjoud L, Keller JM, Schohn H, Dauca M** 2003 Induction of the expression of the peroxisome proliferator-activated receptor alpha (PPARalpha) by clofibrate in jerboa tissues. *Microsc Res Tech* 61:185-90
  54. **Horton JD, Goldstein JL, Brown MS** 2002 SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 109:1125-31
  55. **Yu S, Matsusue K, Kashireddy P, Cao WQ, Yeldandi V, Yeldandi AV, Rao MS, Gonzalez FJ, Reddy JK** 2003 Adipocyte-specific gene expression and adipogenic steatosis in the mouse liver due to peroxisome proliferator-activated receptor gamma1 (PPARgamma1) overexpression *J Biol Chem* 278:498-505
  56. **Millet L, Vidal H, Larrouy D, Andreelli F, Laville M, Langin D** 1998 mRNA expression of the long and short forms of uncoupling protein-3 in obese and lean humans *Diabetologia* 41:829-32

Table 1: jerboa treatment conditions

group	A	Ph	PhC	H
n =	10	10	10	10
3 weeks with food <i>ad libitum</i>	$22 \pm 2$ °C	$22 \pm 2$ °C	$22 \pm 2$ °C	$22 \pm 2$ °C
3 weeks with food <i>ad libitum</i>	$22 \pm 2$ °C	$6 \pm 2$ °C	$6 \pm 2$ °C	$6 \pm 2$ °C
food withdrawal	sacrificed	sacrificed	sacrificed	$6 \pm 2$ °C
				$6 \pm 2$ °C
				4 days hibernation
				sacrificed

## Figure Legends:

Figure 1: Fasting-associated hibernation induction of hepatic PPAR $\alpha$  and PGC-1 $\alpha$  and their target genes ACOX1 and PMP70. (Left panel) Representative RNA analysis performed by RT-PCR of RNA from active (A), prehibernating (P), hibernating (H), and prehibernating ciprofibrate-treated (PhC) jerboas. The signal of  $\beta$ -actin is shown as a housekeeping control gene. (Right panel) Bars represent mean of fold-activation compared with the values of active jerboas, based on densitometric analysis. All values are normalized to  $\beta$ -actin. Letters *a*, *b*, *c*, *d*, *e*, and *f* correspond to a statistical significance of higher mean signal intensity, ( $p < 0.01$  for *a* and *d*,  $p < 0.02$  for *b* and *c*,  $p < 0.05$  for *f*), compared with the active control jerboas (*a* and *b*) or with the prehibernating jerboas (*d*, *e* and *f*). The values are based on 3 independent experiments. A.U.: arbitrary units.

Figure 2: A. Tissue expression analysis of 52 kDa wild type PPAR $\alpha$  (PPAR $\alpha$ 1wt) and 29 kDa truncated PPAR $\alpha$  (PPAR $\alpha$ 2tr) forms performed by Western blotting (50  $\mu$ g proteins) from liver, kidney, heart, muscle, and brain of euthermic active jerboas (A) using a monoclonal antibody against the N-terminal peptide of human PPAR $\alpha$ .

B. Fasting-associated hibernation regulation of wild type PPAR $\alpha$  (PPAR $\alpha$ 1wt) and truncated PPAR $\alpha$  (PPAR $\alpha$ 2tr) proteins analyzed by Western blotting (lower panel) (50  $\mu$ g proteins) from active (A), prehibernating (P), hibernating (H), or prehibernating ciprofibrate-treated (PhC) jerboas. The signal of GAPDH is shown as a housekeeping control gene. (Upper panel) Bars represent mean of log fold-activation of the ratio compared with active animals. Black bars represent the ratio of PPAR $\alpha$ 1wt to GAPDH values, and white bars represent the ratio of PPAR $\alpha$ 2tr to GAPDH values. All values are normalized to GAPDH based on densitometric analysis. \* denotes a significantly ( $P < 0.05$ ) higher mean signal intensity compared with active jerboas.

Figure 3: Fasting-associated hibernation has a strong effect on the expression of ACOX1, catalase, L-PBE, and PMP70 proteins encoded by PPAR $\alpha$ -target genes. Western blotting analysis of protein (50  $\mu$ g) homogenates from euthermic active (A), prehibernating (Ph), hibernating (H), and prehibernating ciprofibrate-treated (PhC) Jerboas. The molecular weights in kDa of the 2 ACOX1 polypeptides (72 and 52 kDa) are indicated. The signal of  $\beta$ -actin is shown as a housekeeping control gene.

Figure 4: Hibernation-dependent regulation and ciprofibrate effect on peroxisomal and mitochondrial fatty acid oxidation. A. Fold-activation of peroxisomal ACOX1 (left) and catalase (right) activities. B. Fold-activation of mitochondrial CPT1 (white bars) and MCAD (dark bars) activities. Bars represent mean values  $\pm$  standard deviations of percent fold-activation compared with the active animals of at least 3 different animals. The specific activities were measured in crude liver extracts of euthermic active (A), prehibernating (Ph), hibernating (H), or prehibernating ciprofibrate-treated (PhC) jerboas as described in the Material and Methods. Letters *a*, *b*, *c*, *d* and *e* correspond to a statistical significance of higher mean signal intensity, ( $p < 0.01$  for *a* and *d*,  $p < 0.02$  for *b* and *e*,  $p < 0.05$  for *c*), compared with the active control jerboas (*a*, *b* and *c*) or with the prehibernating jerboas (*d* and *e*). Values ( $\pm$  standard deviations) of the specific enzymatic activities of active jerboas are: ACOX 182  $\pm$  14 pmol/min/mg, catalase 51.5  $\pm$  8.9  $\mu$ mol/min/mg, MCAD 37.7  $\pm$  6.1 nmol/min/mg, and CPT1 19.8  $\pm$  1.5 nmol/min/mg.

Figure 5: Model of the hibernation-dependent splicing of primary PPAR $\alpha$  transcript in response to fasting conditions of jerboas. The expression of PPAR $\alpha$ 2tr isoform, during prehibernation, prevents the full activation of PPAR $\alpha$  target genes involved in the peroxisomal fatty acids oxidation. The absence of the expression of PPAR $\alpha$ 2tr, during hibernation, increases the availability of PPAR $\alpha$ 1wt active isoform, which, in the presence of PGC1 $\alpha$  coactivator, strongly induces the oxidation of fatty acids in the peroxisomal compartment.



Figure1:

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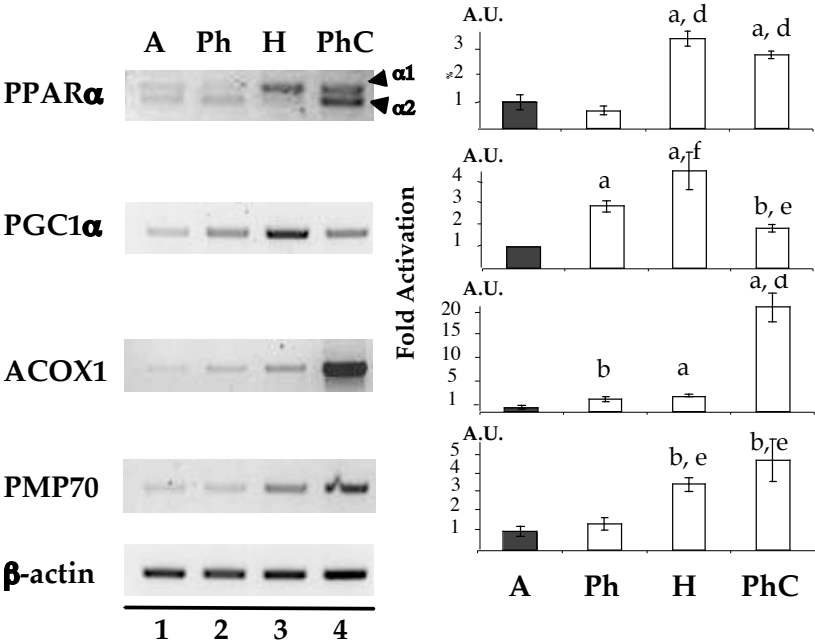


Figure 2:

MS#EN-07-0913

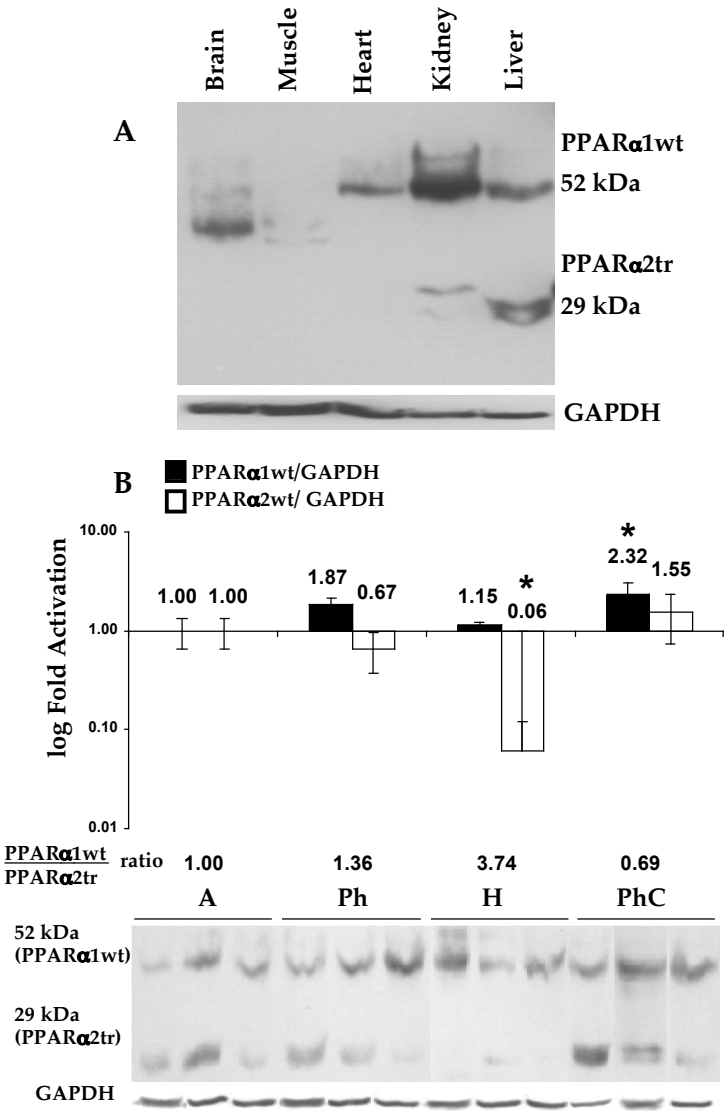


Figure 3:

MS#EN-07-0913

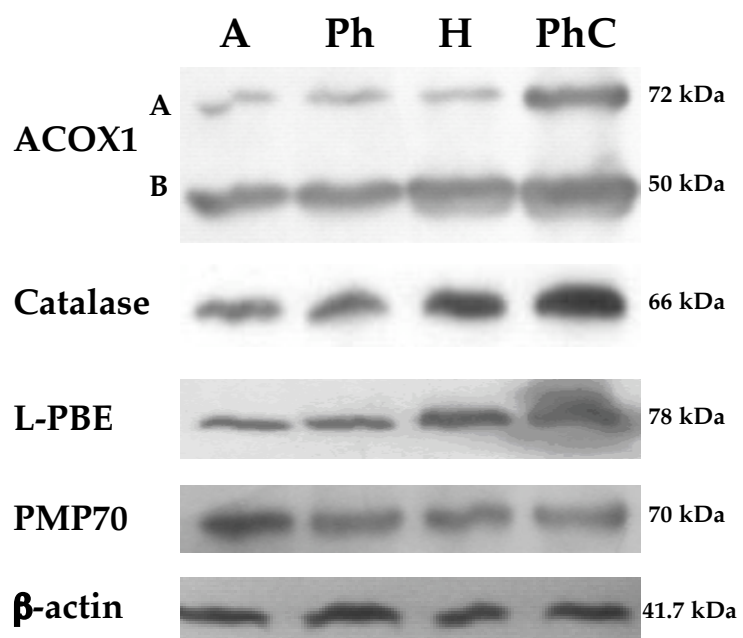


Figure 4:

MS#EN-07-0913

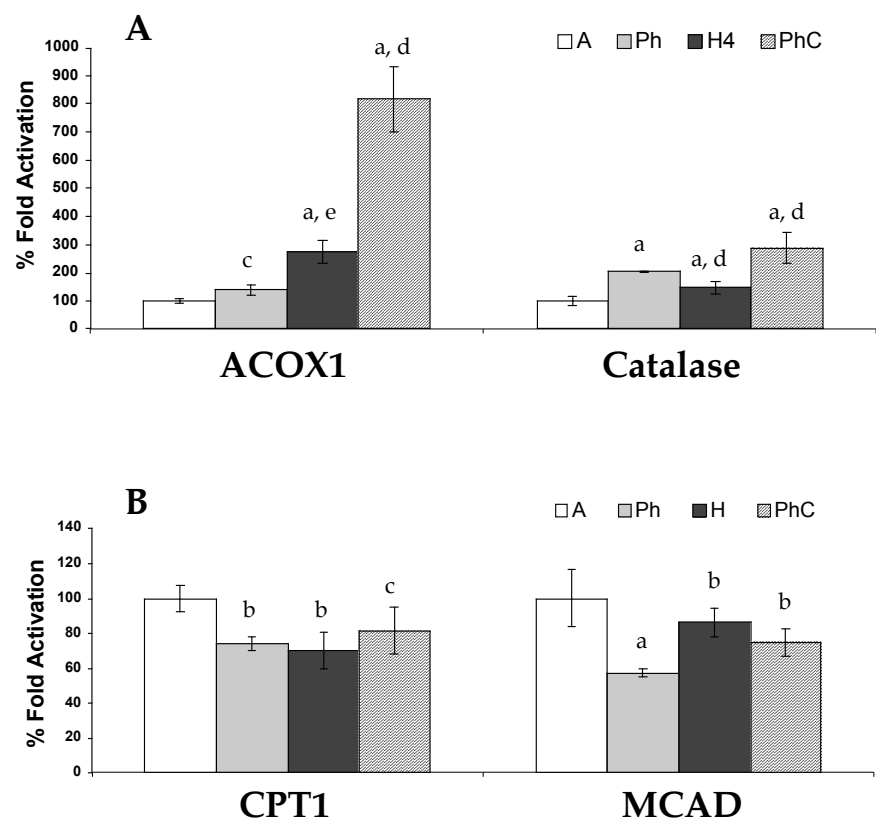
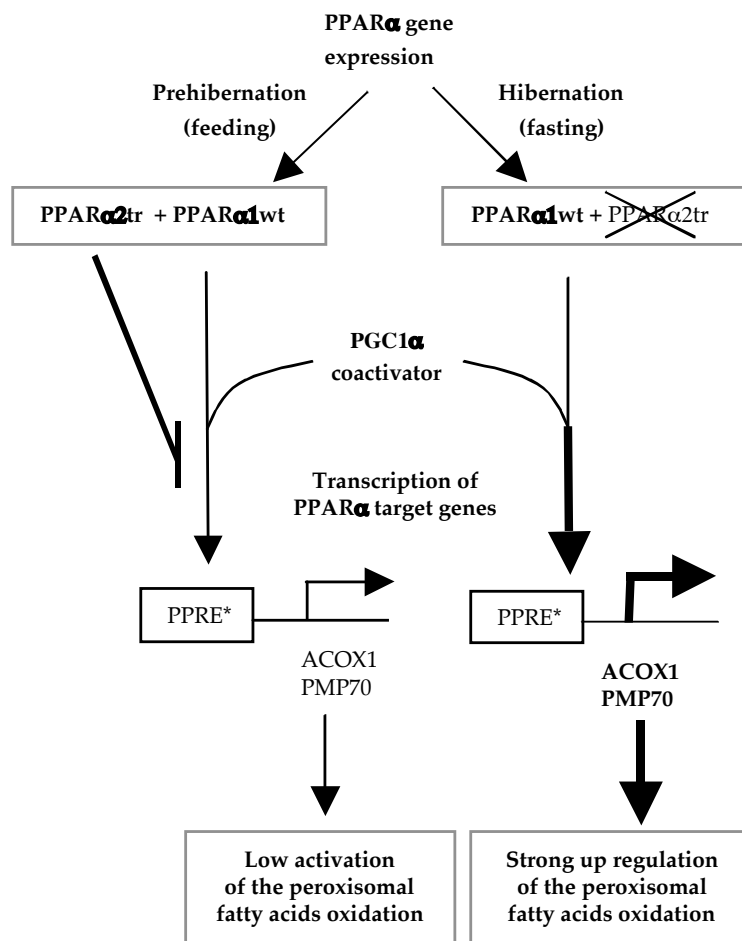


Figure 5:

MS#EN-07-0913



(\*PPRE = PPAR Response Element)