Peroxisomal Changes During Hiberation of Jerboa (Jaculus orientalis)

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ABSTRACT: As a member of the order of Rodentia, jerboa (Jaculus orientalis) is a natural deep hibernator and lives in subdesert highland in many parts of the world, including Morocco. Its small size (adult body weight ~100 g), availability in the wild, tolerance to laboratory conditions, and some unique peroxisomal properties make it a suitable research subject for exploring peroxisome biogenesis under prehibernating and hibernating states. During 3 w, animals referred to as the prehibernator group were exposed to cold temperature (5 to 7°C) with food *ad libitum*. Part of the prehibernator group entered deep hibernation 24 to 48 h after starvation. Animals were sacrificed 4 and 6 d after starting hibernation. As a control, a third group, consisting of active animals, was maintained at 22°C. Concerning hibernation, results from plasma analysis showed an increased level for both ketonemia and ureamia, while triglyceredemia was decreased. Liver acyl-CoA oxidase activity, a peroxisomal b-oxidation enzyme, increased during hibernation. Liver peroxisomal urate oxidase was induced only during the prehibernating state and remained at an increased level until the fourth day of hibernation. The variations were concomitant to a decrease in peroxisomal protein yield and a differential change in peroxisomal protein pattern in sodium dodecyl sulfate-polyacrylamide gel electrophoresis during prehibernating or hibernating states. These preliminary results show that cold exposure and hibernation affect biogenesis of liver peroxisomes in jerboa.
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KEY WORDS: Acyl-CoA dehydrogenase, Acyl-CoA oxidase, hibernation, jerboa, peroxisomes, urate oxidase.

Hibernation and torpor are adaptive strategies that several mammal and bird species use to survive food scarcity and low ambient temperature. Under such inhospitable conditions, hibernation helps some mammals, particularly small ones, to pass the winter in a torpid or lethargic state, which is characterized by low body temperature and depressed metabolism. Survival to extreme low body temperature and ability to rewarm from such a low temperature require a special adaptation of cell metabolism. These physiological changes require a strict control of lipid metabolism, which plays a critical role in supplying the energy requirements during hibernation.

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Previous studies on peroxisomes have shown that cold adaptation provokes remarkable biochemical and ultrastructural changes in rat tissues, i.e., liver and brown fat (1–4). In the cold-adapted rat, nonshivering thermogenesis was greatly increased in response to low ambient temperature (3,5). Strong increases in tissue metabolism and heat production were observed in the rat brown adipose tissue, concomitant with induction of peroxisomal β-oxidation (2). Peroxisomes, single membrane-bound organelles that contain H_2O_2-producing oxidases and H_2O_2-decomposing catalase, are known to be the sites for β-oxidation of long-chain fatty acids, as well as for ether-lipid biosynthesis (6), and play a key role in lipid homeostasis. Interestingly, the first step in the peroxisomal β-oxidation system does not carry provision for the conservation of energy and is a particularly wasteful form of respiration (7,8). However, this peroxisomal respiration could be energetically useful for the cell during cold adaptation.

Recently, we described that jerboa (*Jaculus orientalis*) liver exhibits a higher palmitoyl-CoA oxidase activity than the rat liver (9), showing that peroxisomal β-oxidation system could play a key role in lipid metabolism of jerboa liver. Indeed, jerboa is considered a true hibernator (10) and increases its fat mass during autumn, the prehibernating period, and hibernates in winter. Thus, this peculiar physiological adaptation of jerboa could represent an interesting model to study peroxisomal biogenesis under hibernation conditions.

Therefore, the primary aim of the present work was to explore the temporal response of liver peroxisomal oxidative capacity by following peroxisomal activities and protein content during the hibernation process.

MATERIALS AND METHODS

Young adult jerboa (*J. orientalis*) between 4 and 6 mon of age (110–140 g body weight) were captured in the subdesert of eastern Morocco. In France, they were preacclimatized in the laboratory for 3 wk at 22 ± 2°C with food (rat diet biscuits; Aliments UAR-Villemoisson, Orge, France) and water *ad libitum.* For prehibernating and hibernating states, a group of 10 animals (3 to 4 per cage) was kept with food in a cold room (6°C) for 3 wk. This group was called the prehibernator group (PH). A second group was kept under the same conditions as PH except that the food contained ciprofibrate at a dosage of

3 mg kg/day, and was called the ciprofibrate treated-prehibernator (PHC). The third group was housed as PH and at the end of three weeks of prehibernation period the food was removed. Hibernation was soon established and groups of animals were killed at the fourth and the sixth days of hibernation. The last group was referred to as euthermic animals, called (A).

Plasma analysis : cholesterolemia (total cholesterol), glycemia (glucose), ketonemia (D-3-hydroxybutyrate), triglyceridemia (glycerol) and ureanemia (urea) were done by the Laboratoire de Biochimie Médicale, CHU le Bocage, Dijon, France.

Liver mitochondria and peroxisomes isolation : mitochondria were isolated according to de Duve et al.(11). Peroxisomes were purified according to Ghosh and Hajra (12), using a 30% Nycodenz step gradient centrifugation. Protein content was estimated with the Bio-rad assay according to Bradford (13), using bovine serum albumin as a standard.

SDS-PAGE : The solubilized proteins from purified mitochondrial or peroxisomal fractions were separated on 10% SDS-PAGE (14).

Enzyme assays : measurement of enzyme activities in subcellular fractions were assayed according to the following methods : acyl-CoA dehydrogenase (15) from mitochondria, cyanide insensitive palmitoyl-CoA oxidase and uricase for peroxisomes (16).

Results :

Plasma parameter analysis : Glycemia : during the cold period of prehibernation, jerboa shown no change in glycemia. After four days (H4) of hibernation plasmatic glucose was decreased by 30% and two days later (H6, six days hibernation) decreased by more than 70% as compared to the control (A) (Fig 1A).

Ketone bodies : no significant changes were observed under the prehibernation state. (However, by the fourth day of hibernation plasma b-hydroxybutyrate increased by 30% while it increased 15 fold after six days of hibernation (Fig. 1B)).

Ureamia : in prehibernating conditions, plasma urea increased slightly by 50% and the same value was recorded after four days of hibernation. At the opposite, a six days hibernation (H6) shows a 7-fold increase in urea concentration (Fig. 1C).

No significant effect of both prehibernating and hibernating states on plasma cholesterol level (Fig. 1D) were observed, whereas the triacylglycerol concentration was reduced by 80% after 6 days of hibernation (Fig. 1E).

Under ciprofibrate treatment, jerboa do not show any significant effect on plasma concentrations of the different measured paremeters.

Somatic index : cold exposure of jerboa shows a 24% decrease of liver mass as compared to euthermic animals (table I). This mass decrease is llesser during hibernation(-15%). Furthermore, somatic index of both heart and kidney were not significantly modified in hibernation period (Table I).

Protein content : An increase in liver mitochondrial yield was shown after exposure of the jerboa to cold. During prehibernation, mitochondrial level increased by 47%, while during hibernation no significant change of mitochondrial proteins yield were observed (Table I).

In contrast to mitochondrial protein level, isolated peroxisomes show a strong decrease (about 60% as compared to the control) in both prehibernating and hibernating states. Jerboa treatment with ciprofibrate, had a positive effect during prehibernation showing a 60% and a 35% increases of protein level in mitochondria and in peroxisomes respectively (Table I).

Peroxisomal and mitochondrial b-oxidation: To follow the effect of cold on peroxisomal b-oxidation, the activity of cyanide-insensitive palmitoyl-CoA oxidase, a rate limiting b-oxidation enzyme was measured during prehibernation. A slight but not significant decrease was seen in jerboa liver peroxisomes, while during hibernation the peroxisomal activity increased by 75% (Fig. 2A).

Mitochondrial b-oxidation, as measured by acyl-CoA dehydrogenase activity, showed a 50% decrease in prehibernating animals, whereas a slight increase 25% was recorded during hibernation (Fig. 2B).

Ciprofibrate has a different effect on mitochondrial and on peroxisomal b-oxidation systems. During cold exposure, ciprofibrate increases palmitoyl-CoA oxidase 4-fold (Fig. 2A) while mitochondrial acyl-CoA dehydrogenase activity is decreased by 25% (Fig. 2B).

Uricase (urate: oxygen oxidoreductase) activity : Cold provokes a two-fold increase in liver jerboa peroxisome enzyme activity, and the level was maintained during hibernation. As for palmitoyl-CoA oxidase, ciprofibrate treatment increased urate oxidase activity 3 fold(Fig. 2C).

SDS-PAGE : electrophoretic pattern shows a differential expression of peroxisomal and mitochondrial proteins in SDS-PAGE after coomassie blue staining. Jerboa exposure to cold conditions (5-7° C) indicated a new pattern of polypeptides with enhanced density staining for mitochondrial proteins (figure 3A): a high molecular weight (MW) (150 kDa) and middle MW (60, 45 and 30 kDa), and for peroxisomal proteins (figure 3B): 90, 66, 50, 35 and 20 kDa. This pattern was similar in hibernating animals, but not for all bands, for example, the 35 kDa increases in cold animal except after 6 days hibernation. This polypeptide has the similar molecular weight as uricase (36 kDa) and shows similar variation as its activity during prehibernation and the first four days of hibernation (H4), but this level returns to normal value at the day

six of hibernation (H6).

Discussion :

Hypoglycemia has been reported in several species during hibernation (dormice, hedgehogs and arctic ground squirrels) (17). Elsewhere, hypoglycemia has been observed in hibernating jerboa (18). Here we show that the reduction in plasma glucose concentration is progressive and concomitant during hibernation.

In the animal, there is a decline in gluconeogenesis over a long period of fasting. Gluconeogenesis would exclusively occur in the liver, as this organ still has an active glucose-6-phosphatase (19). Although, like fatty acids, glucose serves as a energy source, thus explains a decline in plasma glucose.

Lipid utilization is an oxidative process, under which fatty acids degrade releasing ketone bodies as the end products. During fasting, the ketone bodies serve as a fuel for peripheral organs, such as the brain and heart (20). Indeed, at low glucose plasma concentrations, an increase in ketone bodies was shown in dormant jerboa, and may play a key role in tissue energy supply. Comparatively, a significant increase of ketone bodies was observed in cold adapted rat (3).

In mammals, urea synthesis is the main pathway to eliminate ammonia, a toxic agent for the central nervous system. Furthermore, a fasting during hibernation, leads to increased protein turnover which participates in urea formation. Nelson (21) suggests that in hibernation the nitrogen product is recaptured by ammonia fixation to a-ketoglutaric acid yielding glutamic acid. On the other hand, D-amino acid oxidase activity is highly induced in liver of cold adapted rats (3). This enhanced activity may contribute to essential liver thermogenesis and production of ketoacids and ammonia, which can be metabolized or converted to urea. The present study shows that plasma urea reaches a 7 fold increase at the sixth day in hibernating jerboa, which may suggest an increased protein catabolism in the animal.

Previous studies have shown an increase in peroxisomes population in both brown fat (2) and in liver (3, 4) from rat adapted to cold exposure. An increase (+ 50%) in numerical density of liver peroxisomes was observed (3). This change was accompanied by reduction of the peroxisome average volume (from 0.27 to 0.19 µm3) and thus the small peroxisome population, also called microperoxisomes, is induced in rat liver (+ 78%) after only one day exposure to cold (4). In contrast, our results show a 60% decrease of peroxisomal proteins yield (mg/g liver), after purification of peroxisomes on 30% Nycodenz cushion from the mitochondrial light fraction (10 000g pellet). In our experiment, this discrepancy may be explained, taking into account that a decrease in peroxisome size implies a shift in peroxisome density, which modifies sedimenting properties (4). Thus a newly synthesized microperoxisomes may sediment with the microsomes fraction (4). However, it is noteworthy that the first period of cold exposure of jerboa (3 weeks) corresponds to a prehibernating state, which is physiologically different from rat cold acclimatation.

Euthermic jerboa respond to ciprofibrate treatment, a hypolipemic agent, by increasing moderately, the peroxisomal protein yield by 35% (9). Cold exposure produced a similar increase, showing that low ambiant temperture has no effect on ciprofibrate action in term of liver peroxisomal proteins content.

There is now evidence on the presence of distinct peroxisomal b-oxidation system from the mitochondrial one, from yeast to mammals (6). These two b-oxidation machineries take place in liver cells as shown by Hryb and Hogg (15), where mitochondrial b-oxidation is more active towards short and medium chains of acyl-CoA, whereas the peroxisomal system is more specific to long and very long chain acyl-CoA. Recently, we have shown that jerboa liver exhibits a higher palmitoyl-CoA oxidase activity as compared to rat liver activity (9). Cold exposure during 3 weeks, had no effect on jerboa liver palmitoyl-CoA oxidase, while during the first week of hibernation, this peroxisomal activity increased by 75%. This delayed cold effect was also demonstrated in rat liver during rat cold exposure (3, 4).

In contrast to peroxisomal acyl-CoA oxidase, the mitochondrial acyl-CoA dehydrogenase displays a 50% decrease. The contribution of mitochondria to whole liver fatty acids b-oxidation is 70 to 90% of the total pathway (6). Negative regulation of mitochondrial b-oxidation may account in the weak lipolysis urate in jerboa liver during prehibernating state, where animals accumulate fat to forsee hibernating period and arousal that need a high lipolysis to supply energy (18).

An increase in liver palmitoyl-CoA oxidase activity under ciprofibrate treatment has been shown before (9). Here, similar results were obtained at low ambient temperature with an increase in peroxisomal protein bulk. A peroxisome proliferator, induces an atrophy of rat brown adipose tissue (22). Furthermore clofibrate feeding leads to a reduced fatty acid synthesis rate in brown fat but neither in liver nor in white adipose tissue (23). In the same way, ciprofibrate treatment of jerboa may interfere with the key role of brown adipose tissue in thermogenesis and enhance liver peroxisomal b-oxidation during prehibernating period in which animal fat accumulation is needed for these animals.

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Legend of figures :

Figure 1: Measurement of plasmatic parameters : glucose A., ketone bodies B., urea C., cholesterol D. and triglyceride E. from active jerboa : A, prehibernating jerboa for 3 weeks: PH, prehibernating jerboa treated with ciprofibarte for 3 weeks : PHC, hibernating jerboa 4 days: H4 and hibernating 6 days: H6. Number in parentheses indicates the fold variation as compared to A group.Value are given as means ± standard deviation of 3 to 4 animals per group..

Figure 2: Peroxisomal palmitoyl-CoA oxidase A., mitochondrial palmitoyl-CoA dehydrogenase B. and peroxisomal urate oxidase C. activities were measured in liver homogenate from active jerboa : A, prehibernating jerboa for 3 weeks: PH, prehibernating jerboa treated with ciprofibarte for 3 weeks : PHC, hibernating jerboa 4 days: H4 and hibernating 6 days: H6.
Number in parentheses indicates the fold variation as compared to A group. Value are given as means ± standard deviationof 3 to 4 animals per group..

Figure 3 : SDS-PAGE of liver mitochondrial A. and peroxisomal B. polypeptides from active jerboa : A, prehibernating jerboa for 3 weeks: PH, prehibernating jerboa treated with ciprofibarte for 3 weeks : PHC, hibernating jerboa 4 days: H4 and hibernating 6 days: H6. The arrow Æ. indicates the molecular weight of polypeptide which vary according to the jerboa treatment.